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EFFICACY AND SAFETY OF ABORTION VACCINES PREPARED FROM BRUCELLA ABORTUS STRAINS OF DIFFERENT DEGREES OF VIRULENCE [1]

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INTRODUCTION

A considerable amount of research has been done during the last quarter of a century to gain more definite information on the value of biologics in controlling infectious abortion. Some of the results reported have stimulated hope that biological prophylaxis may eventually be improved to such a degree that it will serve a useful purpose in the control of Bang's disease. Other results have been less favorable and in some instances have even created doubt as to whether methods of combating the disease based on artificial immunization will ever be developed to a point where confidence can be placed in them.

Favorable results in general have appeared to be confined to efforts to immunize by the use of abortion vaccines (suspensions of living *Brucella abortus*). Such vaccines, when prepared from virulent strains of *Br. abortus*, have commonly been administered only to nonpregnant animals on the theory that when subcutaneously administered to such animals the vaccinal infection would be overcome in a short time. Since they are incapable of conferring the disease, vaccines prepared from avirulent strains of *Br. abortus* have been administered to both pregnant and nonpregnant animals.

As investigations of artificial immunization progressed, it was discovered that all nonpregnant animals do not resist or promptly overcome the vaccinal infection in their bodies when virulent strains are employed in the preparation of the vaccines. This fact was indicated by agglutination tests of blood and examinations of the milk of vaccinated animals. The danger of implanting *Brucella abortus* for prolonged periods by vaccination has seemed to be greater in cows with functioning udders than in virgin heifers.

The possibility of causing an increased number of carriers of the abortion microorganism by vaccination has not been pleasing to contemplate, especially since it has been discovered that *Brucella abortus* may cause undulant fever in man.

The use of avirulent strains in the preparation of vaccines would seem to eliminate the objectionable feature. It remains to be determined, however, whether strains that have lost their disease-producing characteristics may not also have lost much of their efficacy as immunizing agents. The possibility of this has already been indicated, at least in some measure, by results of experiments made at the

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Experiment Station in 1926 and 1928 with a small number of cattle.[2] It was with the object of acquiring more definite information as to the comparative immunizing value of different strains of *Br. abortus* and their freedom from objectionable effects, a feature that had previously received limited consideration in a vaccination experiment with calves,[3] that the experiment reported in this paper was undertaken.

VACCINES USED AND TESTS FOR VIRULENCE

The vaccines used in this experiment were prepared from three different *Brucella abortus* (bovine) strains, numbered 11, 19, and 484, representing three degrees of virulence. Strain 11 had been propagated on artificial culture media for 12 years; strain 19, for 6 years; and strain 484, for only 6 months.

The method used for estimating the degree of virulence possessed by each strain consisted in inoculating guinea pigs both subcutaneously and intra-abdominally with suspensions of the strains, killing the animals after about nine weeks, examining them for indications of *Brucella abortus* lesions, and making agglutination tests of their blood sera taken at the time of autopsy. Table 1 shows the results of inoculations obtained with vaccines prepared from strains 11, 19, and 484, the dosages and intervals between inoculation and autopsy being similar.

Although the guinea pigs inoculated with vaccine from strain 11 yielded reasonably marked agglutination reactions, they did not show visible lesions of *Brucella abortus* infection at the time of autopsy. The guinea pigs inoculated with vaccine from strain 19 gave more marked serological reactions and a few animals showed moderately marked *Br. abortus* lesions. All the guinea pigs which received strain 484 gave pronounced serological reactions and showed extensive *Br. abortus* lesions.

[2] COTTON, W. E. EFFICACY OF DIFFERENT STRAINS OF BRUCELLA ABORTUS AS IMMUNIZING AGENTS AGAINST INFECTIOUS ABORTION. Jour. Agr. Research 45: 705–724. 1932.

[3] BUCK, J. M. STUDIES OF VACCINATION DURING CALFHOOD TO PREVENT BOVINE INFECTIOUS ABORTION. Jour. Agr. Research 41: 667–689. 1930.

TABLE 1.—*Guinea-pig-inoculation results with three strains of Brucella abortus vaccine*

STRAIN 11 VACCINE (GROWN ON CULTURE MEDIUM FOR 12 YEARS)

Guinea pig No.	Site of injection	Date of injection, 1929	Date animal was killed, 1929	Agglutination results at serum dilutions indicated [a]					Autopsy findings
				1:25	1:50	1:100	1:200	1:500	
7788	Abdomen	Apr. 23	July 1	+	+	+	−	−	No lesions.
7789	do	do	do	+	+	+	−	−	Do.
7790	do	do	do	+	+	−	−	−	Do.
7791	do	do	do	+	−	−	−	−	Do.
7792	do	do	do	+	+	+	−	−	Do.
7794	Subcutis	do	do	+	+	+	−	−	Do.
7795	do	do	do	+	+	+	−	−	Do.
7796	do	do	do	+	+	+	+	−	Do.
7797	do	do	do	+	+	+	−	−	Do.
7798	do	do	do	+	+	P	−	−	Do.
7799	do	do	do	+	−	−	−	−	Do.
7900	do	do	do	P	−	−	−	−	Do.
7901	do	do	do	+	+	+	+	+	Do.
7902	do	do	do	+	+	+	+	−	Do.
7903	do	do	do	+	+	−	−	−	Do.
7904	do	do	do	+	+	+	+	+	Do.
7905	do	do	do	+	+	+	−	−	Do.

STRAIN 19 VACCINE (GROWN ON CULTURE MEDIUM FOR 6 YEARS)

7740	Abdomen	Apr. 23	June 25	+	+	+	P	−	No lesions.
7741	do	do	do	+	+	+	+	+	Do.
7742	do	do	do	+	+	+	+	−	Carpal joints slightly enlarged.
7743	do	do	do	+	+	+	P	−	No lesions.
7744	do	do	do	+	+	+	+	−	Spleen slightly enlarged; testicles atrophied.
7745	do	do	do	+	+	+	+	+	Testicles atrophied.
7758	Subcutis	do	do	+	+	+	−	−	No lesions.
7759	do	do	do	+	+	+	+	+	Do.
7760	do	do	do	+	+	+	+	S	Do.
7761	do	do	do	−	−	−	−	−	Do.
7762	do	do	do	+	S	−	−	−	Do.
7763	do	do	do	+	+	−	−	−	Do.
7764	do	do	do	+	+	+	+	+	Do.
7765	do	do	do	+	+	+	+	+	Do.
7766	do	do	do	+	+	+	+	+	Spleen enlarged.
7768	do	do	do	+	+	+	−	−	No lesions.
7769	do	do	do	+	+	+	+	+	Do.

STRAIN 484 VACCINE (GROWN ON CULTURE MEDIUM FOR 6 MONTHS)

7783	Abdomen	Apr. 23	July 3	+	+	+	+	+	Extensive lesions.
7784	do	do	do	+	+	+	+	+	Do.
7786	do	do	do	+	+	+	+	+	Do.
7787	do	do	do	+	+	+	+	+	Do.
7906	Subcutis	do	do	+	+	+	+	+	Do.
7907	do	do	do	+	+	+	+	+	Do.
7908	do	do	do	+	+	+	+	+	Do.
7909	do	do	do	+	+	+	+	+	Do.
7910	do	do	do	+	+	+	+	+	Do.
7911	do	do	do	+	+	+	+	+	Do.
7912	do	do	do	+	+	+	+	+	Do.
7913	do	do	do	+	+	+	+	+	Do.
7914	do	do	do	+	+	+	+	+	Do.
7915	do	do	do	+	+	+	+	+	Do.
7916	do	do	do	+	+	+	+	+	Do.
7917	do	do	do	+	+	+	+	+	Do.

[a] Key: +=complete agglutination; P=partial agglutination; S=trace of agglutination; and −=no agglutination.

EXPERIMENT ANIMALS AND METHODS OF VACCINATION

Thirty-six cows and heifers, all of which were entirely negative to the agglutination test for Bang's disease, were used in the experiment. Three cows and six heifers received strain 11 vaccine; 3 cows and 6

heifers received strain 19; 3 cows and 6 heifers received strain 484; and 9 heifers were reserved as controls.

In the preparation of the vaccines, the strains were propagated on potato-agar slants in 18 mm tubes. The transfers were incubated for 72 hours, after which the growths were washed off the medium with physiological saline solution. Twenty cubic centimeters of saline was used for removing the growth from each tube.

To determine whether the size of dose was important in conferring immunity, different sized doses of vaccine were used. Four animals in each of the first three groups received, subcutaneously, the growth from 1 potato-agar slant suspended in 20 c c of saline. This vaccine represented a density of about ten times that of tube 1 of the McFarland nephelometer. The same number of animals received in the same manner 10 c c of this vaccine diluted with 10 c c of saline. In all cases half the dose was injected under the skin in front of each shoulder rather than at one point in order to prevent, if possible, severe swellings at the points of injection. The one remaining animal in each group of principals received intradermically one-fourth c c of vaccine of the same density as that of the undiluted vaccine which was administered subcutaneously. Each of the vaccinated animals received but a single treatment.

The vaccines were used on the day they were prepared and were proved to be viable by the inoculation of cultures and of guinea pigs.

Temperatures of the vaccinated cattle were taken just before the administration of the vaccines and on numerous days thereafter to determine whether the degree of immunity conferred bore any relation to the temperature reactions. The thermic reactions appear in Table 2.

In general the temperatures appeared to rise gradually for three days after vaccination. Then they commenced to subside and three days later were again normal.

On the first and second days after vaccination, swellings which occurred at the points of inoculation in general were small, but on the third day most of them were from 2 to 3 inches in diameter and from one-half to three-fourths inch thick. After the third day they gradually diminished in size.

The vaccine from strain 484 seemed to produce more local disturbance than that from either strain 11 or strain 19. In two animals receiving strain 484, there were indications of abscess formation at points of injection, although evacuation of abscess contents was never observed. At no time during the week after vaccination did the animals appear to be lacking in desire for feed.

Breeding was commenced two months after vaccination, but some difficulty was experienced in getting all the animals with calf. The trouble was not confined wholly to the vaccinated stock but was experienced with the controls as well. It was necessary to substitute other heifers for several of the original controls. The breeding difficulties were considered to be partly due to the fact that even during the summer months it was necessary to use a good deal of hay instead of green feed as roughage. The keeping of accurate breeding records was interfered with to some extent by the failure of the animals to show signs of oestrus regularly or always to conceive when served, for, to facilitate breeding, the bull was eventually permitted to run with the cattle during the entire day.

TABLE 2.—*Temperatures of cattle immediately preceding injection and on six days after injection with three Brucella abortus strains*

Animal No.	Preinjection temperature on Apr. 23	Postinjection temperature on—					
		Apr. 24	Apr. 25	Apr. 26	Apr. 27	Apr. 29	Apr. 30
GROUP VACCINATED WITH STRAIN 11 VACCINE (OF LOW VIRULENCE)							
Cow:	°F.	°F.	°F.	°F.	°F.	°F.	°F.
1462	102.6	106.2	105.0	106.2	103.0	103.0	100.6
1498	101.6	103.0	101.6	101.8	101.6	101.6	101.6
1500	102.4	104.0	101.8	105.8	103.0	101.8	101.6
Heifer:							
1441	102.6	105.6	105.4	105.2	103.5	103.8	102.4
1492	102.4	105.8	104.8	105.6	103.3	102.0	101.0
1493	102.0	106.0	106.2	106.2	103.8	102.5	101.6
1507	101.6	104.6	104.2	106.0	104.4	102.0	101.2
1533	103.0	106.0	106.2	106.0	104.8	103.8	100.0
1536	102.6	104.6	106.2	106.0	103.8	101.0	101.2
GROUP VACCINATED WITH STRAIN 19 VACCINE (OF MEDIUM VIRULENCE)							
Cow:							
1452	101.8	103.6	103.6	105.2	103.2	101.8	101.2
1490	102.2	102.8	101.6	102.0	101.8	101.6	101.4
1501	102.4	104.6	103.8	105.0	103.2	101.0	101.0
Heifer:							
1451	101.6	107.0	105.8	106.0	103.0	102.4	102.0
1495	102.0	104.6	104.2	105.8	104.4	101.6	101.6
1505	102.0	105.8	106.0	105.4	104.2	101.0	100.8
1529	101.8	103.6	106.0	105.8	104.4	103.2	102.6
1530	101.6	106.0	105.8	105.2	103.2	102.2	101.0
1537	103.0	105.8	105.2	106.0	104.2	102.5	102.0
GROUP VACCINATED WITH STRAIN 484 VACCINE (OF HIGH VIRULENCE)							
Cow:							
1461	103.5	105.8	106.0	105.6	102.2	102.2	101.6
1502	102.0	104.2	104.6	104.0	102.8	102.4	102.0
1504	102.8	104.6	104.8	105.8	101.8	101.2	102.4
Heifer:							
1386	103.0	106.2	106.6	106.4	105.0	103.8	102.2
1396	103.0	104.2	105.2	104.5	102.6	102.2	101.6
1491	101.8	105.8	104.8	105.8	102.8	102.2	101.0
1499	102.8	105.0	105.6	106.0	102.4	102.4	101.4
1528	102.6	106.0	106.0	105.4	102.0	102.4	102.0
1532	102.6	106.0	106.6	106.0	102.0	101.8	101.4

Since each group of cattle that received vaccines prepared from the different *Brucella abortus* strains contained three cows from which milk or other secretions of the udder could be obtained, steps were taken to determine whether the abortion microorganisms were eliminated from this organ after vaccination. It was planned to obtain composite samples of milk at weekly intervals and inject each of four guinea pigs intra-abdominally with 5 c c of each sample. Since none of the cows were being milked regularly it was sometimes difficult to obtain sufficient quantities of material for these inoculations. The first lot of samples was obtained one week after vaccination. The collection and testing of milk or other udder secretions were continued for more than five months.

TESTS FOR BRUCELLA ABORTUS IN UDDER SECRETIONS OF VACCINATED ANIMALS

The guinea pigs inoculated with the udder secretions were killed approximately two months after inoculation. Agglutination tests of their blood sera and the autopsy findings furnished the information on which the presence or absence of *Brucella abortus* in the samples was based. Table 3 presents the inoculation results.

TABLE 3.—*Results of guinea-pig-inoculation tests made with the milk or other udder secretions of nonpregnant cows vaccinated on April 23, 1929*

Cow from which udder secretion was obtained	Guinea-pig results on date indicated [a]											
	Apr. 30	May 7	May 14	May 21	May 28	June 4	June 11	June 18	July 2	Aug. 13	Sept. 21	Oct. 21
COWS VACCINATED WITH STRAIN 11 VACCINE (OF LOW VIRULENCE)												
No. 1462		−	−	−	−	−	−	−	−	−	−	−
No. 1498	−	−	−	+	−	−	−	+	−	−	−	
No. 1500	−	−	−	−	−	−	−	−	−	−	−	−
COWS VACCINATED WITH STRAIN 19 VACCINE (OF MEDIUM VIRULENCE)												
No. 1452		−	−		−		−	−	−	−	−	−
No. 1490	+	+	+	+	+	+	+	+	+	+	+	+
No. 1501	−	−	−	−	−	−	−	−	−	−	−	−
COWS VACCINATED WITH STRAIN 484 VACCINE (OF HIGH VIRULENCE)												
No. 1461					−	+			+	+		
No. 1502	−	−	−	+	+	+	+	+	+	+	+	+
No. 1504	−	+	+	+	+	+	+	+	+	+	+	

[a] − indicates absence of *Br. abortus* infection in guinea pigs; + indicates abortion disease acquired by one or more guinea pigs.

Table 3 shows that the three cows which received the vaccine prepared from the most virulent strain, No. 484, eliminated *Br. abortus* in their milk for an extended period. Cow 1490, which received intradermically one-fourth c c of vaccine prepared from the strain of medium virulence, No. 19, also eliminated the abortion microorganism in her milk as long as these tests were continued, but no evidence was obtained that *Br. abortus* was present in the milk of the other two cows, Nos. 1452 and 1501, which received subcutaneously 10 and 20 c c of vaccine prepared from this strain. The milk of cow 1498, which received intradermically one-fourth c c of the vaccine prepared from strain 11, the least virulent of the three strains used, on two occasions gave evidence of being slightly infectious for guinea pigs. Although the injections of her milk failed to produce visible lesions in the guinea pigs, these injections produced agglutination reactions of medium intensity in some of the pigs on two different dates. In none of the tests was any evidence obtained which indicated that 20 c c of vaccine prepared from strain 11 caused the milk of the other two cows, Nos. 1462 and 1500, to contain the abortion organisms. The results of these studies strongly indicate that intradermic injections of suspensions of *Br. abortus* may result in the implantation of the infection more frequently than subcutaneous injections and would, therefore, be seriously objectionable in vaccination procedures.

EXPOSURE OF EXPERIMENT ANIMALS TO BRUCELLA ABORTUS

The difficulties that were encountered in getting all the vaccinated animals and controls pregnant at about the same time necessitated their exposure to virulent strains of *Brucella abortus* after pregnancy on different dates and to different strains of the infection. Final results were obtained, moreover, on but 23 animals of the vaccinated groups, whereas the number originally entered in the experiment was 27. Two animals in the strain-11 vaccine group, cow 1500 and heifer 1507, and one animal in the strain-19 vaccine group, heifer 1530, failed to become pregnant after many services. Cow 1461 in the strain-484 vaccine group died five months after vaccination. The cause of death could not be definitely determined.

Thirteen principals and five controls were subjected to *Brucella abortus* exposure on October 19, 1929; 4 principals and 1 control, on February 24, 1930; 5 principals and 1 control, on June 10, 1930; and 1 principal and 1 control, on October 22, 1930. One or more animals treated with each lot of vaccine were included among the number which received virulent *Br. abortus* exposure on each occasion except the last.

The method of exposure employed to determine the degree of immunity which the vaccine conferred was a departure from the methods commonly used in vaccine experiments. From three to four drops of a suspension of *Brucella abortus*, approximately 20 times the density of that of tube 1 of the McFarland nephelometer, was deposited on the conjunctiva of one eye of each animal. Since different animals were exposed on four different dates, it was important that suspensions of equal virulence be employed. In preparing the suspension for the first lot of animals, three strains of *Br. abortus* recently isolated from aborted fetuses were used. The suspensions represented the second, fourth, and fifth transfers, respectively, of these strains. For the second lot of animals, a single recently isolated strain of *Br. abortus* was used. This suspension was prepared from the third transfer of this strain. For the third lot, the third transfer of a single strain of *Br. abortus* was used, and for the fourth lot the second transfer of a single strain was employed.

At times of calving or abortion, six guinea pigs were inoculated with emulsions of uterine fluid or placental material that had been emulsified. Each of six guinea pigs was also inoculated with a composite sample of 5 c c of colostrum. In cases of abortion, cultural studies were also made of the fetuses. Table 4 shows the immunizing results with the cows and heifers used in this experiment. The data show that of the 23 vaccinated cows and heifers for which final records were obtained, 21 produced apparently normal calves, whereas of the 8 controls 7 aborted.

Of the seven animals which received strain 11, six produced vigorous calves. *Brucella abortus* was isolated from the uterus and colostrum of one of the animals (No. 1533) and from the uterus of another (No. 1536), which died while giving birth to a calf. *Brucella abortus* was not isolated from the uterus or colostrum of the other five animals of this group at the time of parturition.

TABLE 4.—*Immunizing results with cows and heifers injected with vaccine prepared from Brucella abortus strains of different degrees of virulence, the animals later being subjected to artificial exposure to Brucella abortus infected materials*

GROUP VACCINATED WITH STRAIN 11 VACCINE (OF LOW VIRULENCE)

Animal No.	Date of vaccination, 1929	Dose	Date of breeding	Date of exposure	Gestation period	Outcome of pregnancy	Results of uterine examination for *Br. abortus*	Results of colostrum examination for *Br. abortus*
Cow:		*C c*			*Days*			
1462	Apr. 23	20	Aug. 31, 1929 Dec. 23, 1929	Feb. 24, 1930	277	Vigorous calf	Negative	Negative.
1498	do	.25	July 11, 1929	Oct. 19, 1929	274	do	do	Do.
Heifer:								
1441	do	20	Aug. 1, 1929	do	282	do	do	Do.
1492	do	10	Aug. 17, 1929 Jan. 9, 1930	Feb. 24, 1930	278	do	do	Do.
1493	do	20	Mar. 26, 1930	June 10, 1930	278	do	do	Do.
1533	do	20	Aug. 8, 1929	Oct. 19, 1929	265	do	Positive	Positive.
1536	do	10	(a)	do		Dam died during calving.	do	

GROUP VACCINATED WITH STRAIN 19 VACCINE (OF MEDIUM VIRULENCE)

Cow:								
1452	Apr. 23	20	June 20, 1929	Oct. 19, 1929	279	Vigorous calf	Negative	Negative.
1490	do	.25	June 25, 1929 July 23, 1929	do	281	do	Positive	Positive.
1501	do	10	June 12, 1929	do	272	do	do	Negative.
Heifer:								
1451	do	20	July 27, 1929	do	275	do	do	Do.
1495	do	10	June 17, 1929	do	278	do	Negative	Do.
1505	do	20	Nov. 1, 1929	Feb. 24, 1930	274	do	do	Do.
1529	do	10	Jan. 29, 1930 Apr. 12, 1930	June 10, 1930	278	do	do	Do.
1537	do	10	(a)	Oct. 19, 1929		do	do	Do.

GROUP VACCINATED WITH STRAIN 484 VACCINE (OF HIGH VIRULENCE)

Cow:								
1502	Apr. 23	0.25	June 14, 1929 July 9, 1929	Oct. 19, 1929	288	Vigorous calf	Negative	Positive.
1504	do	10	June 13, 1929 July 11, 1929 Oct. 15, 1929 Nov. 6, 1929	Feb. 24, 1930	364	Weak calf	Positive	Do.
Heifer:								
1386	do	20	(a)	Oct. 22, 1930		Vigorous calf	Negative	Negative.
1396	do	10	(a)	Oct. 19, 1929		do	do	Do.
1491	do	20	July 20, 1929 Aug. 10, 1929 Oct. 31, 1929 Dec. 7, 1929 Jan. 10, 1930 Feb. 8, 1930	June 10, 1930	271	do	do	Do.
1499	do	10	June 21, 1929	Oct. 19, 1929	280	do	do	Do.
1528	do	20	Feb. 10, 1930	June 10, 1930	283	do	do	Do.
1532	do	10	Mar. 18, 1930	do	272	do	do	Do.

CONTROLS, NOT VACCINATED

Heifer:								
1427			May 27, 1929	Oct. 19, 1929	275	Vigorous calf	Positive	Positive.
1429			June 6, 1929	do	218	Abortion	do	Do.
1447			May 8, 1929 June 21, 1929	do	158	do	do	Do.
1489			June 21, 1929	do	177	do	do	Do.
1548			(a)	do		do	do	Do.
1446			Sept. 3, 1929	Feb. 24, 1930	195	do	do	Do.
1625			Apr. 2, 1930	June 10, 1930	157	do	do	Do.
1584			Dec. 5, 1929 Apr. 3, 1930 July 16, 1930	Oct. 22, 1930	181	do	do	Do.

[a] No record.

The eight animals of the second group, which received strain 19, gave birth to eight vigorous calves. *Brucella abortus* was isolated from the uterus and colostrum of animal 1490 and from the uterus only of animals 1501 and 1451.

The animals of the third group, which received strain 484, produced one weak calf and seven vigorous calves. *Brucella abortus* was isolated at the time of calving from the uterus and colostrum of animal 1504, which produced the weak calf. It was also isolated from the colostrum of animal 1502, but it was not isolated from the uterus or colostrum of any of the other animals of this group.

Of the eight animals in the control group, one produced a vigorous calf. *Brucella abortus* was isolated from the uterus and colostrum of all of the eight at the time of calving or aborting.

In no instance was the placenta retained by any of the vaccinated animals, except No. 1536, which died while expelling what appeared to be a full-term fetus. The placenta was retained by five of the eight controls.

Detailed records of the individual animals for which either calving or aborting data were obtained are shown in Table 5.

TABLE 5.—*Records of individual experimental animals* [a]

VACCINATED COW 1462

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used [b]						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine (*Brucella abortus* strain 11).
Apr. 23, 1929	−	−	−	−	−	−	
Apr. 30, 1929	+	+	+	+	+	+	Apr. 30, 1929, milk negative for *Br. abortus*.
May 7, 1929	+	+	+	+	+	+	May 7, 1929, milk negative for *Br. abortus*.
May 14, 1929	+	+	+	+	+	+	May 14, 1929, milk negative for *Br. abortus*.
May 21, 1929	+	+	+	+	P	−	May 21, 1929, milk negative for *Br. abortus*.
June 4, 1929	+	+	+	+	−	−	May 28, 1929, milk negative for *Br. abortus*.
June 18, 1929	+	+	+	S	−	−	June 4, 1929, milk negative for *Br. abortus*.
July 2, 1929	+	+	+	P	−	−	June 11, 1929, milk negative for *Br. abortus*.
July 17, 1929	+	+	+	P	−	−	June 18, 1929, milk negative for *Br. abortus*.
July 31, 1929	+	+	+	−	−	−	July 2, 1929, milk negative for *Br. abortus*.
Aug. 27, 1929	+	+	+	−	−	−	Aug. 13, 1929, milk negative for *Br. abortus*.
Sept. 11, 1929	+	+	−	−	−	−	Aug. 31, 1929, bred.
Sept. 24, 1929	+	+	+	−	−	−	Sept. 21, 1929, milk negative for *Br. abortus*.
Oct. 8, 1929	+	+	−	−	−	−	Oct. 21, 1929, milk negative for *Br. abortus*.
Oct. 21, 1929	+	P	−	−	−	−	
Nov. 6, 1929	+	S	−	−	−	−	
Nov. 19, 1929	+	+	−	−	−	−	
Dec. 19, 1929	+	+	S	−	−	−	
Jan. 8, 1930	+	+	−	−	−	−	Dec. 23, 1929, bred.
Feb. 5, 1930	P	−	−	−	−	−	
Mar. 5, 1930	+	+	−	−	−	−	Feb. 24, 1930, received *Br. abortus*, conjunctival exposure.
Mar. 19, 1930	+	+	+	−	−	−	
Apr. 2, 1930	+	+	S	−	−	−	
Apr. 22, 1930	+	+	P	−	−	−	
May 17, 1930	+	+	+	S	−	−	
June 17, 1930	+	+	+	S	−	−	
June 28, 1930	+	+	+	P	−	−	
July 16, 1930	+	+	+	P	−	−	
Aug. 19, 1930	+	+	+	S	−	−	Sept. 26, 1930, produced a vigorous calf (277 days); guinea pigs inoculated with uterine material and colostrum failed to acquire abortion disease.
Oct. 2, 1930	+	+	+	−	−	−	
Nov. 11, 1930	+	+	P	−	−	−	

[a] Explanation of symbols: The plus sign indicates complete clumping of bacteria; P, partial clumping; S, trace of agglutination; minus sign, no evidence of clumping.

[b] Figures at head of columns indicate cubic centimeters of blood serum. They represent approximate dilutions of 1 to 25, 1 to 50, 1 to 100, 1 to 200, 1 to 500, and 1 to 1,000, respectively, with the amount of antigen (1 c c) used.

TABLE 5.—*Records of individual experimental animals*—Continued

VACCINATED COW 1498

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929	−	−	−	−	−	−	Apr. 2₀, 1929, intradermic injection of ¼ c c abortion vaccine (*Br. abortus* strain 11).
Apr. 23, 1929	−	−	−	−	−	−	
Apr. 30, 1929	+	+	+	P	−	−	Apr. 30, 1929, milk negative for *Br. abortus*.
May 7, 1929	+	+	+	+	+	+	May 7, 1929, milk negative for *Br. abortus*.
May 14, 1929	+	+	+	+	+	+	May 14, 1929, milk negative for *Br. abortus*.
May 21, 1929	+	+	+	+	+	P	May 21, 1929, milk positive for *Br. abortus*.
June 4, 1929	+	+	+	+	+	+	May 28, 1929, milk negative for *Br. abortus*.
June 18, 1929	+	+	P	P	P	P	June 4, 1929, milk negative for *Br. abortus*.
July 2, 1929	+	+	+	+	+	+	June 11, 1929, milk negative for *Br. abortus*.
July 17, 1929	+	+	+	+	+	−	June 18, 1929, milk positive for *Br. abortus*.
July 31, 1929	+	+	+	P	−	−	July 2, 1929, milk negative for *Br. abortus*.
Aug. 27, 1929	+	+	+	+	S	−	July 11, 1929, bred.
Sept. 11, 1929	+	+	+	+	−	−	Aug. 13, 1929, milk negative for *Br. abortus*.
Sept. 24, 1929	+	+	+	+	−	−	Sept. 21, 1929, milk negative for *Br. abortus*.
Oct. 8, 1929	+	+	P	P	−	−	Oct. 19, 1929, received *Br. abortus*, conjunctival exposure.
Oct. 21, 1929	+	+	+	S	−	−	
Nov. 6, 1929	+	+	+	+	−	−	Oct. 21, 1929, milk negative for *Br. abortus*.
Nov. 19, 1929	+	+	P	P	−	−	
Dec. 19, 1929	+	+	+	+	−	−	
Jan. 8, 1930	+	+	+	+	−	−	
Jan. 22, 1930	+	+	+	−	−	−	
Feb. 5, 1930	+	+	+	+	−	−	
Feb. 19, 1930	+	+	+	+	−	−	
Mar. 5, 1930	+	+	+	+	−	−	
Mar. 19, 1930	+	+	P	−	−	−	
Apr. 2, 1930	+	+	+	P	−	−	Apr. 11, 1930, produced a vigorous calf (274 days); guinea pigs inoculated with uterine material and colostrum did not acquire abortion disease.
Apr. 16, 1930	+	+	+	P	−	−	
July 16, 1930	+	+	+	+	−	−	
Aug. 19, 1930	+	+	+	P	−	−	Aug. 19, 1930, milk negative for *Br. abortus*.

VACCINATED HEIFER 1441

Apr. 10, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine (*Br. abortus* strain 11).
Apr. 23, 1929	−	−	−	−	−	−	
Apr. 30, 1929	+	+	+	+	+	+	
May 7, 1929	+	+	+	+	+	+	
May 14, 1929	+	+	+	+	+	+	
May 21, 1929	+	+	+	+	+	+	
June 4, 1929	+	+	+	+	+	−	
June 18, 1929	+	+	+	+	−	−	
July 2, 1929	+	+	+	+	S	−	
July 17, 1929	+	+	+	+	−	−	
July 31, 1929	+	+	P	−	−	−	
Aug. 27, 1929	+	+	P	−	−	−	Aug. 1, 1929, bred.
Sept. 11, 1929	+	+	+	−	−	−	
Sept. 24, 1929	+	+	+	+	−	−	
Oct. 8, 1929	+	+	+	S	−	−	
Oct. 21, 1929	+	+	+	−	−	−	Oct. 19, 1929, received *Br. abortus*, conjunctival exposure.
Nov. 6, 1929	+	+	+	−	−	−	
Nov. 19, 1929	+	+	+	−	−	−	
Dec. 19, 1929	+	+	S	−	−	−	
Jan. 8, 1930	+	+	−	−	−	−	
Jan. 22, 1930	+	+	+	−	−	−	
Feb. 5, 1930	+	P	−	−	−	−	
Feb. 19, 1930	+	P	−	−	−	−	
Mar. 5, 1930	+	+	−	−	−	−	
Mar. 19, 1930	+	P	−	−	−	−	
Apr. 2, 1930	+	+	S	−	−	−	
Apr. 16, 1930	+	+	−	−	−	−	May 10, 1930, produced a vigorous calf (282 days); guinea pigs inoculated with uterine material and colostrum failed to acquire abortion disease.
Apr. 22, 1930	+	P	−	−	−	−	
July 16, 1930	+	P	−	−	−	−	
Aug. 19, 1930	+	+	P	−	−	−	Aug. 19, 1930, milk negative for *Br. abortus*.

TABLE 5.—*Records of individual experimental animals*—Continued

VACCINATED HEIFER 1492

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929	−	−	−	−	−	−	
Apr. 23, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine (*Br. abortus* strain 11).
Apr. 30, 1929	+	+	+	+	+	+	
May 7, 1929	+	+	+	+	+	+	
May 14, 1929	+	+	+	+	+	+	
May 21, 1929	+	+	+	+	+	+	
June 4, 1929	+	+	+	+	+	+	
June 18, 1929	+	+	+	+	−	P	
July 2, 1929	+	+	+	+	−	−	
July 17, 1929	+	+	+	+	−	−	
July 31, 1929	+	+	+	−	−	−	Aug. 17, 1929, bred.
Aug. 27, 1929	+	+	S	S	−	−	
Sept. 11, 1929	+	+	+	−	−	−	
Sept. 24, 1929	+	+	+	−	−	−	
Oct. 8, 1929	+	+	+	−	−	−	
Oct. 21, 1929	+	+	+	−	−	−	
Nov. 6, 1929	+	+	P	−	−	−	
Nov. 19, 1929	+	+	P	−	−	−	
Dec. 19, 1929	+	+	+	S	−	−	
Jan. 8, 1930	+	+	P	−	−	−	Jan. 9. 1930, bred.
Feb. 5, 1930	+	+	+	−	−	−	Feb. 24, 1930, received *Br. abortus*, conjunctival exposure.
Mar. 5, 1930	+	+	+	+	−	−	
Mar. 19, 1930	+	+	+	S	−	−	
Apr. 2, 1930	+	+	+	S	−	−	
Apr. 16, 1930	+	+	+	−	−	−	
Apr. 22, 1930	+	+	P	−	−	−	
May 7, 1930	+	+	+	−	−	−	
May 17, 1930	+	+	+	−	−	−	
June 28, 1930	+	+	+	S	−	−	
July 16, 1930	+	+	P	P	−	−	Oct. 14, 1930, produced a vigorous calf (278 days);
Aug. 19, 1930	+	+	+	P	−	−	guinea pigs inoculated with uterine material and
Jan. 9, 1931	+	+	+	P	−	−	colostrum failed to acquire abortion disease.

VACCINATED HEIFER 1493

Apr. 10, 1929	−	−	−	−	−	−	
Apr. 23, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine (*Br. abortus* strain 11).
Apr. 30, 1929	+	+	+	+	+	+	
May 7, 1929	+	+	+	+	+	+	
May 14, 1929	+	+	+	+	+	+	
May 21, 1929	+	+	+	+	+	P	
June 4, 1929	+	+	+	+	+	−	
June 18, 1929	+	+	+	+	−	−	
July 2, 1929	+	+	+	−	−	−	
July 17, 1929	+	+	+	P	−	−	
July 31, 1929	+	+	+	−	−	−	
Aug. 27, 1929	+	P	S	−	−	−	
Sept. 11, 1929	P	P	−	−	−	−	
Sept. 24, 1929	+	+	P	−	−	−	
Oct. 8, 1929	+	+	−	−	−	−	
Oct. 21, 1929	+	+	−	−	−	−	
Nov. 6, 1929	P	−	−	−	−	−	
Nov. 19, 1929	+	−	−	−	−	−	
Dec. 19, 1929	+	S	−	−	−	−	
Jan. 8, 1930	−	−	−	−	−	−	
Feb. 5, 1930	+	S	−	−	−	−	
Mar. 5, 1930	+	S	−	−	−	−	Mar. 26, 1930, bred.
Apr. 2, 1930	P	S	−	−	−	−	
May 17, 1930	+	+	P	−	−	−	June 10, 1930, received *Br. abortus*, conjunctival
June 17, 1930	+	+	+	+	−	−	exposure.
June 28, 1930	+	+	+	P	−	−	
July 16, 1930	+	+	+	S	−	−	
Aug. 19, 1930	+	+	S	−	−	−	
Oct. 2, 1930	+	+	+	−	−	−	Dec. 29, 1930, produced a vigorous calf (278 days);
Nov. 11, 1930	+	+	P	−	−	−	guinea pigs inoculated with uterine material
Nov. 22, 1930	P	P	−	−	−	−	and colostrum failed to acquire abortion disease.
Jan. 9, 1931	+	+	+	−	−	−	Mar. 20, 1931, milk negative for *Br. abortus*.

TABLE 5.—*Records of individual experimental animals*—Continued

VACCINATED HEIFER 1533

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929	−	−	−	−	−	−	
Apr. 23, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine (*Br. abortus* strain 11).
Apr. 30, 1929	+	+	+	+	+	+	
May 7, 1929	+	+	+	+	+	−	
May 14, 1929	+	+	+	+	P	−	
May 21, 1929	+	+	+	+	P	−	
June 4, 1929	+	+	+	+	−	−	
June 18, 1929	+	+	+	+	−	−	
July 2, 1929	+	+	+	−	−	−	
July 17, 1929	+	+	P	−	−	−	
July 31, 1929	P	−	−	−	−	−	Aug. 8, 1929, bred.
Aug. 27, 1929	P	S	−	−	−	−	
Sept. 11, 1929	−	−	−	−	−	−	
Sept. 24, 1929	P	−	−	−	−	−	
Oct. 8, 1929	P	−	−	−	−	−	
Oct. 21, 1929	−	−	−	−	−	−	Oct. 19, 1929, received *Br. abortus*, conjunctival exposure.
Nov. 6, 1929	+	+	−	−	−	−	
Nov. 19, 1929	+	+	P	−	−	−	
Dec. 19, 1929	S	−	−	−	−	−	
Jan. 8, 1930	−	−	−	−	−	−	
Jan. 22, 1930	+	+	−	−	−	−	
Feb. 5, 1930	+	−	−	−	−	−	
Feb. 19, 1930	S	−	−	−	−	−	
Mar. 5, 1930	−	−	−	−	−	−	
Mar. 19, 1930	−	−	−	−	−	−	
Apr. 2, 1930	−	−	−	−	−	−	Apr. 30, 1930, produced a vigorous calf (265 days); 4 out of 6 guinea pigs inoculated with uterine material and 1 pig out of 6 inoculated with colostrum acquired abortion disease.
Apr. 16, 1930	−	−	−	−	−	−	
Apr. 22, 1930	−	−	−	−	−	−	
July 16, 1930	−	−	−	−	−	−	
Aug. 19, 1930	−	−	−	−	−	−	Aug. 19, 1930, milk negative for *Br. abortus*.

VACCINATED HEIFER 1536

Apr. 10, 1929	−	−	−	−	−	−	
Apr. 23, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine (*Br. abortus* strain 11).
Apr. 30, 1929	+	+	+	+	+	+	
May 7, 1929	+	+	+	+	+	+	
May 14, 1929	+	+	+	+	+	+	
May 21, 1929	+	+	+	+	+	S	No breeding record.
June 4, 1929	+	+	+	+	−	−	
June 18, 1929	+	+	+	+	−	−	
July 2, 1929	+	+	+	+	−	−	
July 17, 1929	+	+	+	−	−	−	
July 31, 1929	+	+	+	−	−	−	
Aug. 27, 1929	+	P	S	−	−	−	
Sept. 11, 1929	+	+	−	−	−	−	Oct. 19, 1929, received *Br. abortus*, conjunctival exposure.
Sept. 24, 1929	+	+	−	−	−	−	
Oct. 8, 1929	+	+	−	−	−	−	Nov. 29, 1929, dam died while calving; guinea pigs inoculated with uterine material acquired abortion disease. (Dam seemingly vaccinated by mistake when bred.)
Oct. 21, 1929	+	−	−	−	−	−	
Nov. 6, 1929	+	+	+	−	−	−	
Nov. 19, 1929	+	+	−	−	−	−	

TABLE 5.—*Records of individual experimental animals*—Continued

VACCINATED COW 1452

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929	−	−	−	−	−	−	
Apr. 23, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine (*Br. abortus* strain 19).
Apr. 30, 1929	+	+	+	+	+	+	
May 7, 1929	+	+	+	+	+	+	May 7, 1929, milk negative for *Br. abortus*.
May 14, 1929	+	+	+	+	+	+	May 14, 1929, milk negative for Br. *abortus*.
May 21, 1929	+	+	+	+	+	+	May 28, 1929, milk negative for *Br. abortus*.
June 4, 1929	+	+	+	+	+	−	June 11, 1929, milk negative for *Br. abortus*.
June 18, 1929	+	+	+	+	−	−	June 18, 1929, milk negative for *Br. abortus*.
July 2, 1929	+	+	+	P	−	−	June 20, 1929, bred.
July 17, 1929	+	+	+	P	−	−	July 2, 1929, milk negative for *Br. abortus*.
July 31, 1929	+	+	+	−	−	−	
Aug. 27, 1929	+	+	+	−	−	−	Aug. 13, 1929, milk negative for *Br. abortus*.
Sept. 11, 1929	+	+	−	−	−	−	Sept. 21, 1929, milk negative for *Br. abortus*.
Sept. 24, 1929	+	P	P	−	−	−	Oct. 19, 1929, received *Br. abortus*, conjunctival exposure.
Oct. 8, 1929	+	+	P	−	−	−	
Oct. 21, 1929	+	+	−	−	−	−	Oct. 21, 1929, milk negative for *Br. abortus*.
Nov. 6, 1929	+	+	+	−	−	−	
Nov. 19, 1929	+	+	+	+	−	−	
Dec. 19, 1929	+	+	+	−	−	−	
Jan. 8, 1930	+	+	−	−	−	−	
Jan. 22, 1930	+	+	+	−	−	−	
Feb. 5, 1930	+	+	S	−	−	−	
Feb. 19, 1930	+	+	P	−	−	−	
Mar. 5, 1930	+	+	−	−	−	−	
Mar. 19, 1930	+	P	−	−	−	−	Mar. 26, 1930, produced a vigorous calf (279 days); guinea pigs inoculated with uterine material and colostrum failed to acquire abortion disease.
Apr. 2, 1930	+	+	P	−	−	−	
Apr. 16, 1930	+	P	−	−	−	−	
July 16, 1930	+	+	−	−	−	−	

VACCINATED COW 1490

Apr. 10, 1929	−	−	−	−	−	−	Apr. 23, 1929, intradermic injection of ¼ c c abortion vaccine (*Br. abortus* strain 19).
Apr. 23, 1929	−	−	−	−	−	−	
Apr. 30, 1929	+	+	P	P	−	−	Apr. 30, 1929, milk positive for *Br. abortus*.
May 7, 1929	+	+	+	+	+	P	May 7, 1929, milk positive for *Br. abortus*.
May 14, 1929	+	+	+	+	+	+	May 14, 1929, milk positive for *Br. abortus*.
May 21, 1929	+	+	+	+	+	+	May 21, 1929, milk positive for *Br. abortus*.
June 4, 1929	+	+	+	+	+	+	May 28, 1929, milk positive for *Br. abortus*.
June 18, 1929	+	+	+	+	+	+	June 4, 1929, milk positive for *Br. abortus*.
July 2, 1929	+	+	+	+	+	+	June 11, 1929, milk positive for *Br. abortus*.
July 17, 1929	+	+	+	+	+	+	June 18, 1929, milk positive for *Br. abortus*.
July 31, 1929	+	+	+	+	+	+	June 25, 1929, bred.
Aug. 27, 1929	+	+	+	+	+	+	July 2, 1929, milk positive for *Br. abortus*.
Sept. 11, 1929	+	+	+	+	+	P	July 23, 1929, bred.
Sept. 24, 1929	+	+	+	+	+	+	Aug. 13, 1929, milk positive for *Br. abortus*.
Oct. 8, 1929	+	+	+	+	+	+	Sept. 21, 1929, milk positive for *Br. abortus*.
Oct. 21, 1929	+	+	+	+	+	+	Oct. 21, 1929, milk positive for *Br. abortus*.
Nov. 6, 1929	+	+	+	+	+	+	Oct. 19, 1929, received *Br. abortus*, conjunctival exposure.
Nov. 19, 1929	+	+	+	+	+	+	
Dec. 19, 1929	+	+	+	+	+	+	
Jan. 8, 1930	+	+	+	+	+	+	
Jan. 22, 1930	+	+	+	+	+	+	
Feb. 5, 1930	+	+	+	+	+	+	
Feb. 19, 1930	+	+	+	+	+	+	
Mar. 5, 1930	+	+	+	+	+	P	
Mar. 19, 1930	+	+	+	+	+	P	
Apr. 2, 1930	+	+	+	+	+	+	Apr. 30, 1930, produced a vigorous calf (281 days); guinea pigs inoculated with uterine material and colostrum acquired abortion disease.
Apr. 16, 1930	+	+	+	+	+	+	
Apr. 22, 1930	+	+	+	+	+	−	
July 16, 1930	+	+	+	+	+	−	
Aug. 19, 1930	+	+	+	+	+	+	Aug. 16, 1930, milk positive for *Br. abortus*.
Nov. 11, 1930	+	+	+	+	+	+	Aug. 22, 1930, milk positive for *Br. abortus*.
Nov. 22, 1930	+	+	+	+	+	+	

TABLE 5.—*Records of individual experimental animals*—Continued

VACCINATED COW 1501

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929	−	−	−	−	−	−	
Apr. 23, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine (*Br. abortus* strain 19).
Apr. 30, 1929	+	+	+	+	+	+	Apr. 30, 1929, milk negative for *Br. abortus*.
May 7, 1929	+	+	+	+	+	+	May 7, 1929, milk negative for *Br. abortus*.
May 14, 1929	+	+	+	+	+	+	May 14, 1929, milk negative for *Br. abortus*.
May 21, 1929	+	+	+	+	+	+	May 21, 1929, milk negative for *Br. abortus*.
June 4, 1929	+	+	+	+	+	−	June 4, 1929, milk negative for *Br. abortus*.
June 18, 1929	+	+	+	+	+	−	June 11, 1929, milk negative for *Br. abortus*.
July 2, 1929	+	+	+	+	−	−	June 12, 1929, bred.
July 17, 1929	+	+	+	−	−	−	June 18, 1929, milk negative for *Br. abortus*.
July 31, 1929	+	+	+	−	−	−	July 2, 1929, milk negative for *Br. abortus*.
Aug. 27, 1929	+	+	+	−	−	−	Aug. 13, 1929, milk negative for *Br. abortus*.
Sept. 11, 1929	+	+	+	−	−	−	Sept. 21, 1929, milk negative for *Br. abortus*.
Sept. 24, 1929	+	+	+	−	−	−	Oct. 19, 1929, received *Br. abortus*, conjunctival exposure.
Oct. 8, 1929	+	+	+	−	−	−	Oct. 21, 1929, milk negative for *Br. abortus*.
Oct. 21, 1929	+	+	+	−	−	−	
Nov. 6, 1929	+	+	+	+	−	−	
Nov. 19, 1929	+	+	+	+	−	−	
Dec. 19, 1929	+	+	+	+	−	−	
Jan. 8, 1930	+	+	+	P	−	−	
Jan. 22, 1930	+	+	+	P	−	−	
Feb. 5, 1930	+	+	+	+	−	−	
Feb. 19, 1930	+	+	+	P	−	−	
Mar. 5, 1930	+	+	+	−	−	−	Mar. 11, 1930, produced a vigorous calf (272 days); guinea pigs inoculated with uterine material acquired abortion disease, whereas colostrum failed to infect them.
Mar. 19, 1930	+	+	+	−	−	−	
Apr. 2, 1930	+	+	+	−	−	−	
Apr. 16, 1930	+	+	+	−	−	−	
July 16, 1930	+	+	+	P	−	−	
Aug. 19, 1930	+	+	+	S	−	−	Aug. 19, 1930, milk negative for *Br. abortus*.
Oct. 2, 1930	+	+	+	−	−	−	
Nov. 11, 1930	+	P	S	−	−	−	
Nov. 22, 1930	+	+	+	−	−	−	
Jan. 9, 1931	+	+	+	−	−	−	Jan. 24, 1931, milk negative for *Br. abortus*.

VACCINATED HEIFER 1451

Apr. 10, 1929	−	−	−	−	−	−	
Apr. 23, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine (*Br. abortus* strain 19).
Apr. 30, 1929	+	+	+	+	+	+	
May 7, 1929	+	+	+	+	+	+	
May 14, 1929	+	+	+	+	+	+	
May 21, 1929	+	+	+	+	+	+	
June 4, 1929	+	+	+	+	+	−	
June 18, 1929	+	+	+	+	−	−	
July 2, 1929	+	+	+	+	−	−	
July 17, 1929	+	+	+	+	P	−	
July 31, 1929	+	+	+	+	−	−	July 27, 1929, bred.
Aug. 27, 1929	+	+	+	−	−	−	
Sept. 11, 1929	+	+	+	−	−	−	
Sept. 24, 1929	+	+	+	−	−	−	
Oct. 8, 1929	+	+	+	−	−	−	
Oct. 21, 1929	+	+	S	−	−	−	Oct. 19, 1929, received *Br. abortus*, conjunctival exposure.
Nov. 6, 1929	+	+	+	+	−	−	
Nov. 19, 1929	+	+	+	−	−	−	
Dec. 19, 1929	+	+	+	−	−	−	
Jan. 8, 1930	+	+	+	−	−	−	
Jan. 22, 1930	+	+	+	−	−	−	
Feb. 5, 1930	+	+	P	−	−	−	
Feb. 19, 1930	+	+	+	P	−	−	
Mar. 5, 1930	+	+	+	−	−	−	
Mar. 19, 1930	+	+	P	−	−	−	
Apr. 2, 1930	+	+	−	−	−	−	Apr. 28, 1930, produced a vigorous calf (275 days); 2 out of 6 guinea pigs inoculated with uterine material acquired abortion disease; colostrum failed to infect guinea pigs.
Apr. 16, 1930	+	S	−	−	−	−	
Apr. 22, 1930	+	+	P	−	−	−	
July 16, 1930	+	+	+	P	−	−	
Aug. 19, 1930	+	+	+	P	−	−	Aug. 19, 1930, milk negative for *Br. abortus*.

TABLE 5.—*Records of individual experimental animals*—Continued

VACCINATED HEIFER 1495

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929	−	−	−	−	−	−	
Apr. 23, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine (*Br. abortus* strain 19).
Apr. 30, 1929	+	+	+	+	+	+	
May 7, 1929	+	+	+	+	+	+	
May 14, 1929	+	+	+	+	+	+	
May 21, 1929	+	+	+	+	+	+	
June 4, 1929	+	+	+	+	+	−	
June 18, 1929	+	+	+	+	−	−	June 17, 1929, bred.
July 2, 1929	+	+	+	+	−	−	
July 17, 1929	+	+	+	+	−	−	
July 31, 1929	+	+	+	S	−	−	
Aug. 27, 1929	+	+	+	S	−	−	
Sept. 11, 1929	+	P	S	−	−	−	
Sept. 24, 1929	+	+	+	−	−	−	
Oct. 8, 1929	+	+	+	−	−	−	
Oct. 21, 1929	+	+	+	−	−	−	Oct. 19, 1929, received *Br. abortus*, conjunctival exposure.
Nov. 6, 1929	+	+	+	+	−	−	
Nov. 19, 1929	+	+	+	P	−	−	
Dec. 19, 1929	+	+	+	−	−	−	
Jan. 8, 1930	+	+	+	−	−	−	
Jan. 22, 1930	+	+	P	−	−	−	
Feb. 5, 1930	+	+	+	−	−	−	
Feb. 19, 1930	+	+	+	−	−	−	
Mar. 5, 1930	+	+	P	−	−	−	
Mar. 19, 1930	+	+	P	−	−	−	Mar. 22, 1930, produced a vigorous calf (278 days); guinea pigs inoculated with uterine material and colostrum failed to acquire abortion disease.
Apr. 2, 1930	+	+	−	−	−	−	
Apr. 16, 1930	+	+	S	−	−	−	
July 16, 1930	+	+	+	S	−	−	Aug. 19, 1930, milk negative for *Br. abortus*.
Aug. 19, 1930	+	+	P	−	−	−	Oct. 4, 1930, milk negative for *Br. abortus*.

VACCINATED HEIFER 1505

Apr. 10, 1929	−	−	−	−	−	−	
Apr. 23, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine (*Br. abortus* strain 19).
Apr. 30, 1929	+	+	+	+	+	+	
May 7, 1929	+	+	+	+	+	+	
May 14, 1929	+	+	+	+	+	+	
May 21, 1929	+	+	+	+	+	+	
June 4, 1929	+	+	+	+	+	−	
June 18, 1929	+	+	+	+	−	−	
July 2, 1929	+	+	+	+	−	−	
July 17, 1929	+	+	+	+	−	−	
July 31, 1929	+	+	+	−	−	−	
Aug. 27, 1929	+	+	S	−	−	−	
Sept. 11, 1929	+	+	+	P	−	−	
Sept. 24, 1929	+	+	+	−	−	−	
Oct. 8, 1929	+	+	P	S	−	−	
Oct. 21, 1929	+	+	+	−	−	−	
Nov. 6, 1929	+	P	−	−	−	−	Nov. 1, 1929, bred.
Nov. 19, 1929	+	+	P	−	−	−	
Dec. 19, 1929	+	+	P	−	−	−	
Jan. 8, 1930	+	P	S	−	−	−	
Jan. 22, 1930	+	+	P	−	−	−	
Feb. 5, 1930	+	+	P	−	−	−	Feb. 24, 1930, received *Br. abortus*, conjunctival exposure.
Mar. 5, 1930	+	+	+	−	−	−	
Mar. 19, 1930	+	+	P	−	−	−	
Apr. 2, 1930	+	+	+	−	−	−	Aug. 2, 1930, produced a vigorous calf (274 days); guinea pigs inoculated with uterine material and colostrum did not acquire abortion disease.
Apr. 16, 1930	+	+	−	−	−	−	
Apr. 22, 1930	+	+	−	−	−	−	
May 7, 1930	+	+	−	−	−	−	
May 17, 1930	+	+	+	−	−	−	
June 17, 1930	+	+	+	S	−	−	
June 28, 1930	+	+	+	S	−	−	
July 16, 1930	+	+	+	S	−	−	
Aug. 19, 1930	+	+	P	−	−	−	
Oct. 2, 1930	+	P	−	−	−	−	

TABLE 5.—*Records of individual experimental animals*—Continued

VACCINATED HEIFER 1529

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929	−	−	−	−	−	−	
Apr. 23, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine (*Br. abortus* strain 19).
Apr. 30, 1929	+	+	+	+	+	+	
May 7, 1929	+	+	+	+	+	+	
May 14, 1929	+	+	+	+	+	+	
May 21, 1929	+	+	+	+	+	+	
June 4, 1929	+	+	+	+	+	P	
June 18, 1929	+	+	+	+	−	−	
July 2, 1929	+	+	+	+	+	−	
July 17, 1929	+	+	P	P	−	−	
July 31, 1929	+	+	−	−	−	−	
Aug. 27, 1929	+	P	S	−	−	−	
Sept. 11, 1929	+	+	P	−	−	−	
Sept. 24, 1929	+	+	P	−	−	−	
Oct. 8, 1929	+	P	S	−	−	−	
Oct. 21, 1929	+	+	P	−	−	−	
Nov. 6, 1929	+	P	−	−	−	−	
Nov. 19, 1929	+	+	−	−	−	−	
Dec. 19, 1929	+	+	P	−	−	−	
Jan. 8, 1930	+	+	−	−	−	−	Jan. 29, 1930, bred.
Feb. 5, 1930	+	+	S	−	−	−	
Mar. 5, 1930	+	+	S	−	−	−	
Apr. 2, 1930	+	+	−	−	−	−	Apr. 12, 1930, bred.
June 11, 1930	+	+	+	+	−	−	June 10, 1930, received *Br. abortus*, conjunctival exposure.
June 17, 1930	+	+	+	S	−	−	
June 28, 1930	+	+	+	+	S	−	Jan. 15, 1931, produced a vigorous calf (278 days); guinea pigs inoculated with uterine material and with colostrum did not acquire abortion disease.
July 16, 1930	+	+	+	+	−	−	
Aug. 19, 1930	+	+	+	+	−	−	
Oct. 2, 1930	+	+	+	+	−	−	
Nov. 11, 1930	+	+	S	−	−	−	Mar. 20, 1931, milk negative for *Br. abortus*.

VACCINATED HEIFER 1537

Apr. 10, 1929	−	−	−	−	−	−	
Apr. 23, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine (*Br. abortus* strain 19).
Apr. 30, 1929	+	+	+	+	+	+	
May 7, 1929	+	+	+	+	+	+	
May 14, 1929	+	+	+	+	+	+	
May 21, 1929	+	+	+	+	+	−	No breeding record. (Was vaccinated by mistake when about one month pregnant.)
June 4, 1929	+	+	+	+	+	−	
June 18, 1929	+	+	+	−	−	−	
July 2, 1929	+	+	+	−	−	−	
July 17, 1929	+	+	+	−	−	−	
July 31, 1929	+	+	S	−	−	−	
Aug. 27, 1929	+	P	−	−	−	−	
Sept. 11, 1929	+	−	−	−	−	−	
Sept. 24, 1929	+	−	−	−	−	−	
Oct. 8, 1929	+	−	−	−	−	−	
Oct. 21, 1929	P	−	−	−	−	−	Oct. 19, 1929, received *Br. abortus*, conjunctival exposure.
Nov. 6, 1929	+	+	−	−	−	−	
Nov. 19, 1929	+	+	−	−	−	−	
Dec. 19, 1929	+	P	−	−	−	−	Dec. 21, 1929, produced a vigorous calf; guinea pigs inoculated with uterine material and with colostrum did not acquire abortion disease.
Jan. 8, 1930	P	−	−	−	−	−	
Jan. 22, 1930	+	+	−	−	−	−	
Feb. 5, 1930	P	−	−	−	−	−	
Feb. 19, 1930	P	−	−	−	−	−	
Mar. 5, 1930	−	−	−	−	−	−	
Mar. 19, 1930	−	−	−	−	−	−	
Apr. 2, 1930	−	−	−	−	−	−	
Apr. 16, 1930	−	−	−	−	−	−	
July 16, 1930	+	P	S	−	−	−	

TABLE 5.—*Records of individual experimental animals*—Continued

VACCINATED COW 1502

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929	−	−	−	−	−	−	Apr. 23, 1929, intradermic injection of ¼ c c abortion vaccine (*Br. abortus* strain 484).
Apr. 23, 1929	−	−	−	−	−	−	Apr. 30, 1929, milk negative for *Br. abortus*.
Apr. 30, 1929	+	+	P	−	−	−	May 7, 1929, milk negative for *Br. abortus*.
May 2, 1929	+	+	P	−	−	−	May 14, 1929, milk negative for *Br. abortus*.
May 7, 1929	+	+	+	+	+	−	May 21, 1929, milk positive for *Br. abortus*.
May 14, 1929	+	+	+	+	P	−	May 28, 1929, milk positive for *Br. abortus*.
May 21, 1929	+	+	+	+	+	−	June 4, 1929, milk positive for *Br. abortus*.
May 28, 1929	+	+	+	+	+	−	June 11, 1929, milk positive for *Br. abortus*.
June 4, 1929	+	+	+	+	+	−	June 14, 1929, bred.
June 18, 1929	+	+	+	+	+	P	June 18, 1929, milk positive for *Br. abortus*.
July 2, 1929	+	+	+	+	+	P	July 2, 1929, milk positive for *Br. abortus*.
July 17, 1929	+	+	+	+	+	−	July 9, 1929, bred.
July 31, 1929	+	+	+	+	+	+	Aug. 13, 1929, milk positive for *Br. abortus*.
Aug. 27, 1929	+	+	+	+	+	S	Sept. 21, 1929, milk positive for *Br. abortus*.
Sept. 11, 1929	+	+	+	+	+	+	Oct. 21, 1929, milk positive for *Br. abortus*.
Sept. 24, 1929	+	+	+	+	+	+	Oct. 19, 1929, received *Br. abortus*, conjunctival exposure.
Oct. 8, 1929	+	+	+	+	+	+	
Oct. 21, 1929	+	+	+	+	+	+	
Nov. 6, 1929	+	+	+	+	+	+	
Nov. 19, 1929	+	+	+	+	+	+	
Dec. 19, 1929	+	+	+	+	+	+	
Jan. 8, 1930	+	+	+	+	+	+	
Jan. 22, 1930	+	+	+	+	+	+	
Feb. 5, 1930	+	+	+	+	+	+	
Mar. 5, 1930	+	+	+	+	+	+	
Mar. 19, 1930	+	+	+	+	+	+	
Apr. 2, 1930	+	+	+	+	+	+	Apr. 23, 1930, produced a vigorous calf (288 days); guinea pigs inoculated with uterine material failed to acquire abortion disease; guinea pigs were infected with abortion disease by colostrum injections.
Apr. 16, 1930	+	+	+	+	+	+	
Apr. 22, 1930	+	+	+	+	+	+	
July 16, 1930	+	+	+	+	+	+	
Aug. 19, 1930	+	+	+	+	+	+	Aug. 16, 1930, milk positive for *Br. abortus*.
Nov. 11, 1930	+	+	+	+	+	+	Aug. 22, 1930, milk positive for *Br. abortus*.
Nov. 22, 1930	+	+	+	+	+	+	Jan. 26, 1931, milk positive for *Br. abortus*.
Jan. 9, 1931	+	+	+	+	+	+	

VACCINATED COW 1504

Apr. 10, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine (*Br. abortus* strain 484).
Apr. 23, 1929	−	−	−	−	−	−	Apr. 30, 1929, milk negative for *Br. abortus*.
Apr. 30, 1929	+	+	+	+	−	−	May 7, 1929, milk positive for *Br. abortus*.
May 2, 1929	+	+	+	+	+	−	May 14, 1929, milk positive for *Br. abortus*.
May 7, 1929	+	+	+	+	+	+	May 21, 1929, milk positive for *Br. abortus*.
May 14, 1929	+	+	+	+	+	+	May 28, 1929, milk positive for *Br. abortus*.
May 21, 1929	+	+	+	+	+	+	June 4, 1929, milk positive for *Br. abortus*.
May 28, 1929	+	+	+	+	+	+	June 13, 1929, bred.
June 4, 1929	+	+	+	+	+	+	June 11, 1929, milk positive for *Br. abortus*.
June 18, 1929	+	+	+	+	+	+	June 18, 1929, milk positive for *Br. abortus*.
July 2, 1929	+	+	+	+	+	+	July 2, 1929, milk positive for *Br. abortus*.
July 17, 1929	+	+	+	+	+	+	July 11, 1929, bred.
July 31, 1929	+	+	+	+	+	+	Aug. 13, 1929, milk positive for *Br. abortus*.
Aug. 27, 1929	+	+	+	+	+	+	Sept. 21, 1929, milk positive for *Br. abortus*.
Sept. 11, 1929	+	+	+	+	+	+	Oct. 15, 1929, bred.
Sept. 24, 1929	+	+	+	+	+	+	Oct. 21, 1929, milk positive for *Br. abortus*.
Oct. 8, 1929	+	+	+	+	+	+	Nov. 6, 1929, bred.
Oct. 21, 1929	+	+	+	+	+	+	
Nov. 6, 1929	+	+	+	+	+	+	
Nov. 19, 1929	+	+	+	+	+	+	
Dec. 19, 1929	+	+	+	+	+	+	
Jan. 8, 1930	+	+	+	+	+	+	Feb. 24, 1930, received *Br. abortus*, conjunctival exposure.
Jan. 22, 1930	+	+	+	+	+	+	
Feb. 5, 1930	+	+	+	+	+	+	
Mar. 5, 1930	+	+	+	+	+	+	
Apr. 2, 1930	+	+	+	+	+	+	
Apr. 16, 1930	+	+	+	+	+	+	July 28, 1930, produced a weak calf (264 days); guinea pigs which were inoculated both with uterine material and colostrum became affected with abortion disease.
Apr. 22, 1930	+	+	+	+	+	+	
May 7, 1930	+	+	+	+	+	+	
May 17, 1930	+	+	+	+	+	+	
June 17, 1930	+	+	+	+	+	+	
June 28, 1930	+	+	+	+	+	+	
July 16, 1930	+	+	+	+	+	+	
Aug. 19, 1930	+	+	+	+	+	+	Aug. 16, 1930, milk positive for *Br. abortus*.
Nov. 11, 1930	+	+	+	+	+	+	Aug. 22, 1930, milk positive for *Br. abortus*.
Nov. 22, 1930	+	+	+	+	+	+	
Jan. 9, 1931	+	+	+	+	+	+	Jan. 24, 1931, milk positive for *Br. abortus*.

TABLE 5.—*Records of individual experimental animals*—Continued

VACCINATED HEIFER 1386

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929	−	−	−	−	−	−	
Apr. 23, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine (*Br. abortus* strain 484).
Apr. 30, 1929	+	+	+	+	+	P	
May 2, 1929	+	+	+	+	+	P	
May 7, 1929	+	+	+	+	+	+	
May 14, 1929	+	+	+	+	+	+	
May 21, 1929	+	+	+	+	+	+	
June 4, 1929	+	+	+	+	P	−	No breeding record.
June 18, 1929	+	+	+	+	+	P	
July 2, 1929	+	+	+	+	+	+	
July 17, 1929	+	+	+	+	+	−	
July 31, 1929	+	+	+	+	−	−	
Aug. 27, 1929	+	+	P	S	−	−	
Sept. 11, 1929	+	+	+	+	−	−	
Sept. 24, 1929	+	+	+	+	−	−	
Oct. 8, 1929	+	+	+	+	−	−	
Oct. 21, 1929	+	+	P	−	−	−	
Nov. 6, 1929	+	+	+	−	−	−	
Nov. 19, 1929	+	+	+	−	−	−	
Dec. 19, 1929	+	S	−	−	−	−	
Jan. 8, 1930	+	P	−	−	−	−	
Feb. 5, 1930	P	P	−	−	−	−	
Mar. 5, 1930	+	+	P	−	−	−	Mar. 3, 1931, produced a vigorous calf; guinea pigs inoculated with uterine material and colostrum did not acquire abortion disease.
Apr. 2, 1930	+	+	+	−	−	−	
Apr. 16, 1930	+	+	+	−	−	−	
July 16, 1930	+	+	+	+	−	−	
Aug. 19, 1930	+	+	+	+	−	−	
Sept. 30, 1930	+	+	+	P	−	−	
Oct. 22, 1930	+	+	P	S	−	−	Oct. 22, 1930, received *Br. abortus*, conjunctival exposure.
Nov. 22, 1930	+	+	+	+	+	−	
Feb. 19, 1931	+	+	+	−	−	−	
June 2, 1931	+	+	+	−	−	−	

VACCINATED HEIFER 1396

Apr. 10, 1929	−	−	−	−	−	−	
Apr. 23, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine (*Br. abortus* strain 484).
May 2, 1929	+	+	+	+	+	P	
May 7, 1929	+	+	+	+	+	+	
May 14, 1929	+	+	+	+	+	+	
May 21, 1929	+	+	+	+	+	+	
June 4, 1929	+	+	+	+	+	−	No breeding record.
June 18, 1929	+	+	+	+	+	+	
July 2, 1929	+	+	+	+	+	+	
July 17, 1929	+	+	+	+	+	−	
July 31, 1929	+	+	+	+	+	−	
Aug. 27, 1929	+	+	+	S	−	−	
Sept. 11, 1929	+	+	+	−	−	−	
Sept. 24, 1929	+	+	+	−	−	−	
Oct. 8, 1929	+	+	+	−	−	−	
Oct. 21, 1929	+	+	P	−	−	−	Oct. 19, 1929, received *Br. abortus*, conjunctival exposure.
Nov. 6, 1929	+	+	+	−	−	−	
Nov. 19, 1929	+	+	+	−	−	−	
Dec. 19, 1929	+	+	+	−	−	−	
Jan. 8, 1930	+	+	P	−	−	−	
Jan. 22, 1930	+	+	+	S	−	−	
Feb. 5, 1930	+	+	+	−	−	−	
Feb. 19, 1930	+	+	+	−	−	−	
Mar. 5, 1930	+	+	P	−	−	−	Apr. 11, 1930, produced a vigorous calf; guinea pigs inoculated with uterine material and guinea pigs inoculated with colostrum did not acquire abortion disease.
Mar. 19, 1930	+	+	+	−	−	−	
Apr. 2, 1930	+	+	−	−	−	−	
July 16, 1930	+	+	P	−	−	−	

TABLE 5.—*Records of individual experimental animals*—Continued

VACCINATED HEIFER 1491

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929	−	−	−	−	−	−	
Apr. 23, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine (*Br. abortus* strain 484).
Apr. 30, 1929	+	+	+	+	+	+	
May 2, 1929	+	+	+	+	+	+	
May 7, 1929	+	+	+	+	+	+	
May 14, 1929	+	+	+	+	+	+	
May 21, 1929	+	+	+	+	+	+	
June 4, 1929	+	+	+	+	+	+	
June 18, 1929	+	+	+	+	+	−	
July 2, 1929	+	+	+	+	+	−	
July 17, 1929	+	+	+	+	+	−	July 20, 1929, bred.
July 31, 1929	+	+	+	+	−	−	
Aug. 27, 1929	+	+	S	−	−	−	Aug. 10, 1929, bred.
Sept. 11, 1929	+	+	+	−	−	−	
Sept. 24, 1929	+	+	+	−	−	−	
Oct. 8, 1929	+	+	+	−	−	−	
Oct. 21, 1929	+	+	+	−	−	−	Oct. 31, 1929, bred.
Nov. 6, 1929	+	+	+	−	−	−	
Nov. 19, 1929	+	+	S	−	−	−	
Dec. 19, 1929	+	+	+	S	−	−	Dec. 7, 1929, bred.
Jan. 8, 1930	+	+	+	P	−	−	Jan. 10, 1930, bred.
Feb. 5, 1930	+	+	+	+	−	−	Feb. 8, 1930, bred.
Mar. 5, 1930	+	+	P	−	−	−	
Apr. 2, 1930	+	+	+	−	−	−	
Apr. 16, 1930	+	+	+	−	−	−	
June 11, 1930	+	+	+	P	S	−	June 10, 1930, received *Br. abortus*, conjunctival exposure.
June 17, 1930	+	+	+	P	−	−	
June 28, 1930	+	+	+	+	−	−	
July 16, 1930	+	+	+	+	−	−	
Aug. 19, 1930	+	+	+	S	−	−	Nov. 6, 1930, produced a vigorous calf (271 days); guinea pigs inoculated with uterine material and with colostrum did not acquire abortion disease.
Oct. 2, 1930	+	+	+	−	−	−	
Nov. 11, 1930	+	+	+	−	−	−	
Nov. 22, 1930	+	+	P	−	−	−	
Jan. 9, 1931	+	+	P	−	−	−	Jan. 28, 1931, milk negative for *Br. abortus*.

VACCINATED HEIFER 1490

Apr. 10, 1929	−	−	−	−	−	−	
Apr. 23, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine (*Br. abortus* strain 484).
Apr. 30, 1929	+	+	+	+	+	P	
May 2, 1929	+	+	+	+	+	−	
May 7, 1929	+	+	+	+	+	+	
May 14, 1929	+	+	+	+	+	+	
May 21, 1929	+	+	+	+	P	−	
June 4, 1929	+	+	+	+	−	−	
June 18, 1929	+	+	+	P	−	−	June 21, 1929, bred.
July 2, 1929	+	+	+	P	−	−	
July 17, 1929	+	+	+	+	−	−	
July 31, 1929	+	+	+	−	−	−	
Aug. 27, 1929	+	+	S	−	−	−	
Sept. 11, 1929	+	+	−	−	−	−	
Sept. 24, 1929	+	+	S	−	−	−	
Oct. 8, 1929	+	P	−	−	−	−	
Oct. 21, 1929	+	+	−	−	−	−	Oct. 19, 1929, received *Br. abortus*, conjunctival exposure.
Nov. 6, 1929	+	+	+	−	−	−	
Nov. 19, 1929	+	+	+	−	−	−	
Dec. 19, 1929	+	+	+	−	−	−	
Jan. 8, 1930	+	+	S	−	−	−	
Jan. 22, 1930	+	+	+	−	−	−	
Feb. 5, 1930	+	+	+	−	−	−	
Feb. 19, 1930	+	+	P	−	−	−	
Mar. 5, 1930	+	+	P	−	−	−	
Mar. 19, 1930	+	+	−	−	−	−	Mar. 28, 1930, produced a vigorous calf (280 days); guinea pigs inoculated with uterine material and guinea pigs inoculated with colostrum failed to acquire abortion disease.
Apr. 2, 1930	+	+	−	−	−	−	
Apr. 16, 1930	+	P	−	−	−	−	
Apr. 22, 1930	+	S	−	−	−	−	
July 16, 1930	+	+	P	−	−	−	
Aug. 19, 1930	+	+	P	−	−	−	Aug. 19, 1930, milk negative for *Br. abortus*.

TABLE 5.—*Records of individual experimental animals*—Continued

VACCINATED HEIFER 1528

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929	−	−	−	−	−	−	
Apr. 23, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine (*Br. abortus* strain 484).
Apr. 30, 1929	P	−	−	−	−	−	
May 2, 1929	+	P	−	−	−	−	
May 7, 1929	+	+	+	+	+	−	
May 14, 1929	+	+	+	+	+	−	
May 21, 1929	+	+	+	+	+	−	
June 4, 1929	+	+	+	+	−	−	
June 18, 1929	+	+	+	P	−	−	
July 2, 1929	+	P	P	−	−	−	
July 17, 1929	+	+	P	−	−	−	
July 31, 1929	+	P	−	−	−	−	
Aug. 27, 1929	P	S	−	−	−	−	
Sept. 11, 1929	+	−	−	−	−	−	
Sept. 24, 1929	+	S	−	−	−	−	
Oct. 8, 1929	+	−	−	−	−	−	
Oct. 21, 1929	P	−	−	−	−	−	
Nov. 6, 1929	P	−	−	−	−	−	
Nov. 19, 1929	P	−	−	−	−	−	
Dec. 19, 1929	P	−	−	−	−	−	
Jan. 8, 1930	P	−	−	−	−	−	
Feb. 5, 1930	S	−	−	−	−	−	Feb. 10, 1930, bred.
Mar. 5, 1930	−	−	−	−	−	−	
Apr. 2, 1930	−	−	−	−	−	−	
June 11, 1930	+	−	−	−	−	−	June 10, 1930, received *Br. abortus*, conjunctival exposure.
June 17, 1930	P	−	−	−	−	−	
June 28, 1930	+	+	+	S	−	−	Nov. 20, 1930, produced a vigorous calf (283 days); guinea pigs inoculated with uterine material and with colostrum did not acquire abortion disease.
July 16, 1930	+	+	+	S	−	−	
Oct. 2, 1930	+	+	−	−	−	−	
Nov. 11, 1930	P	S	−	−	−	−	
Nov. 22, 1930	−	−	−	−	−	−	Jan. 31, 1931, milk negative for *Br. abortus*.

VACCINATED HEIFER 1532

Apr. 10, 1929	−	−	−	−	−	−	
Apr. 23, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine (*Br. abortus* strain 484).
Apr. 30, 1929	+	+	−	−	−	−	
May 2, 1929	+	+	−	−	−	−	
May 7, 1929	+	+	+	+	+	+	
May 14, 1929	+	+	+	+	P	S	
May 21, 1929	+	+	+	+	+	−	
June 4, 1929	+	+	+	+	P	−	
June 18, 1929	+	+	+	+	−	−	
July 2, 1929	+	P	S	−	−	−	
July 17, 1929	P	P	−	−	−	−	
July 31, 1929	−	−	−	−	−	−	
Aug. 27, 1929	P	S	−	−	−	−	
Sept. 11, 1929	S	−	−	−	−	−	
Sept. 24, 1929	P	−	−	−	−	−	
Oct. 8, 1929	−	−	−	−	−	−	
Oct. 21, 1929	P	−	−	−	−	−	
Nov. 6, 1929	−	−	−	−	−	−	
Nov. 19, 1929	−	−	−	−	−	−	
Dec. 19, 1929	−	−	−	−	−	−	
Jan. 8, 1930	P	−	−	−	−	−	
Feb. 5, 1930	−	−	−	−	−	−	
Mar. 5, 1930	−	−	−	−	−	−	Mar. 18, 1930, bred.
Apr. 2, 1930	−	−	−	−	−	−	
Apr. 16, 1930	−	−	−	−	−	−	
June 11, 1930	+	+	−	−	−	−	June 10, 1930, received *Br. abortus*, conjunctival exposure.
June 17, 1930	P	−	−	−	−	−	
June 28, 1930	+	+	P	−	−	−	
July 16, 1930	+	+	+	−	−	−	Dec. 15, 1930, produced a vigorous calf (272 days); guinea pigs inoculated with uterine material and guinea pigs inoculated with colostrum did not acquire abortion disease.
Aug. 19, 1930	P	−	−	−	−	−	
Oct. 2, 1930	−	−	−	−	−	−	
Nov. 11, 1930	S	−	−	−	−	−	
Jan. 9, 1931	S	−	−	−	−	−	Mar. 20, 1931, milk negative for *Br. abortus*.

TABLE 5.—*Records of individual experimental animals*—Continued

CONTROL HEIFER 1427

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Oct. 8, 1929	−	−	−	−	−	−	May 27, 1929, bred.
Oct. 21, 1929	−	−	−	−	−	−	Oct. 19, 1929, received *Br. abortus*, conjunctival exposure.
Nov. 6, 1929	+	P	−	−	−	−	
Nov. 19, 1929	+	+	−	−	−	−	
Dec. 19, 1929	+	+	+	+	−	−	
Jan. 8, 1930	+	+	+	+	+	−	
Jan. 22, 1930	+	+	+	+	+	+	
Feb. 5, 1930	+	+	+	+	+	+	
Feb. 19, 1930	+	+	+	+	+	+	Feb. 26, 1930, produced a vigorous calf (275 days); guinea pigs inoculated with uterine material and guinea pigs inoculated with colostrum acquired abortion disease.
Mar. 5, 1930	+	+	+	+	+	+	
Mar. 19, 1930	+	+	+	+	+	+	
Apr. 2, 1930	+	+	+	+	+	P	
Apr. 16, 1930	+	+	+	+	+	+	
July 16, 1930	+	+	+	+	−	−	
Aug. 19, 1930	+	+	+	P	−	−	Aug. 16, 1930, milk positive for *Br. abortus*.
Oct. 22, 1930	+	+	+	P	−	−	Aug. 22, 1930, milk positive for *Br. abortus*.
Nov. 11, 1930	+	+	P	S	−	−	
Jan. 9, 1931	+	+	+	S	−	−	

CONTROL HEIFER 1429

Oct. 8, 1929	−	−	−	−	−	−	June 6, 1929, bred.
Oct. 21, 1929	−	−	−	−	−	−	Oct. 19, 1929, received *Br. abortus*, conjunctival exposure.
Nov. 6, 1929	+	+	+	+	+	−	
Nov. 19, 1929	+	+	+	+	P	−	
Dec. 19, 1929	+	+	+	+	+	+	
Jan. 8, 1930	+	+	+	+	+	+	Jan. 10, 1930, aborted (218 days); guinea pigs were infected with abortion disease by the inoculation of uterine material and colostrum.
Jan. 22, 1930	+	+	+	+	+	+	
Feb. 5, 1930	+	+	+	+	+	+	
Feb. 19, 1930	+	+	+	+	+	P	
Mar. 5, 1930	+	+	+	+	+	P	
Mar. 19, 1930	+	+	+	+	+	S	

CONTROL HEIFER 1447

Oct. 8, 1929	−	−	−	−	−	−	May 8, 1929, bred.
Oct. 21, 1929	−	−	−	−	−	−	June 21, 1929, bred.
Nov. 6, 1929	+	+	+	S	−	−	Oct. 19, 1929, received *Br. abortus*, conjunctival exposure.
Nov. 19, 1929	+	+	+	+	+	−	
Dec. 19, 1929	+	+	+	+	+	−	
Jan. 8, 1930	+	+	+	+	+	+	Nov. 26, 1929, aborted (158 days); guinea pigs infected with abortion disease by inoculation of uterine material and colostrum.
Jan. 22, 1930	+	+	+	+	+	+	
Feb. 5, 1930	+	+	+	+	+	+	
Mar. 19, 1930	+	+	+	+	+	+	

CONTROL HEIFER 1489

Oct. 8, 1929	−	−	−	−	−	−	June 21, 1929, bred.
Oct. 21, 1929	−	−	−	−	−	−	Oct. 19, 1929, received *Br. abortus*, conjunctival exposure.
Nov. 6, 1929	+	+	P	−	−	−	
Nov. 19, 1929	+	+	+	−	−	−	
Dec. 19, 1929	+	+	+	+	+	+	Dec. 15, 1929, aborted (177 days); *Br. abortus* isolated from uterine material and colostrum through guinea-pig inoculations.
Jan. 8, 1930	+	+	+	+	+	+	
Jan. 22, 1930	+	+	+	+	+	+	
Feb. 5, 1930	+	+	+	+	+	+	
Feb. 19, 1930	+	+	+	+	+	+	
Mar. 5, 1930	+	+	+	+	+	+	
Mar. 19, 1930	+	+	+	+	+	+	
Apr. 2, 1930	+	+	+	+	+	+	
Apr. 16, 1930	+	+	+	+	+	+	
May 7, 1930	+	+	+	+	+	+	
May 17, 1930	+	+	+	+	+	+	
July 16, 1930	+	+	+	+	+	+	
Aug. 19, 1930	+	+	+	+	+	+	

TABLE 5.—*Records of individual experimental animals*—Continued

CONTROL HEIFER 1548

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Oct. 8, 1929	−	−	−	−	−	−	Breeding date not recorded.
Oct. 21, 1929	−	−	−	−	−	−	Oct. 19, 1929, received *Br. abortus*, conjunctival exposure.
Nov. 6, 1929	+	+	+	+	P	−	
Nov. 19, 1929	+	+	+	+	−	−	
Dec. 19, 1929	+	+	+	+	P	−	
Jan. 8, 1930	+	+	+	+	−	−	
Jan. 22, 1930	+	+	+	+	+	−	
Feb. 5, 1930	+	+	+	+	+	−	
Feb. 19, 1930	+	+	+	+	+	+	Feb. 15, 1930, aborted; *Br. abortus* isolated from uterine material and from colostrum through guinea-pig inoculations.
Mar. 5, 1930	+	+	+	+	+	P	
Mar. 19, 1930	+	+	+	+	+	+	
Apr. 2, 1930	+	+	+	+	+	+	
Apr. 16, 1930	+	+	+	+	+	+	
May 7, 1930	+	+	+	+	+	+	
May 17, 1930	+	+	+	+	+	P	
July 16, 1930	+	+	+	+	−	−	
Aug. 19, 1930	+	+	+	+	S	−	

CONTROL HEIFER 1446

Jan. 28, 1930	−	−	−	−	−	−	Sept. 3, 1929, bred.
Mar. 5, 1930	+	+	+	−	−	−	Feb. 24, 1930, received *Br. abortus*, conjunctival exposure.
Mar. 19, 1930	+	+	+	+	+	P	
Apr. 2, 1930	+	+	+	+	+	+	
Apr. 16, 1930	+	+	+	+	+	+	Mar. 17, 1930, aborted (195 days); guinea pigs inoculated with uterine material and with colostrum acquired abortion disease.
May 7, 1930	+	+	+	+	+	+	
July 16, 1930	+	+	+	+	P	P	
Aug. 19, 1930	+	+	+	+	+	+	
Jan. 9, 1931	+	+	+	+	+	+	

CONTROL HEIFER 1625

June 4, 1930	−	−	−	−	−	−	Apr. 2, 1930, bred.
June 10, 1930	−	−	−	−	−	−	June 10, 1930, received *Br. abortus*, conjunctival exposure.
June 17, 1930	P	−	−	−	−	−	
June 28, 1930	+	+	+	+	P	P	
July 16, 1930	+	+	+	+	+	+	
Aug. 19, 1930	+	+	+	+	+	+	
Oct. 2, 1930	+	+	+	+	+	+	Sept. 6, 1930, aborted (157 days); guinea pigs inoculated with uterine material and with colostrum acquired abortion disease.
Nov. 11, 1930	+	+	+	+	+	+	
Jan. 9, 1931	+	+	+	+	+	+	

CONTROL HEIFER 1584

Oct. 2, 1930	−	−	−	−	−	−	Dec. 5, 1929, bred.
Oct. 22, 1930	−	−	−	−	−	−	Apr. 3, 1930, bred.
Nov. 10, 1930	+	+	+	+	P	−	July 16, 1930, bred.
Dec. 20, 1930	+	+	+	+	S	−	Oct. 22, 1930, received *Br. abortus*, conjunctival exposure.
Jan. 9, 1931	+	+	+	+	+	+	
Feb. 19, 1931	+	+	+	+	+	+	Jan. 13, 1931, aborted (181 days); guinea pigs inoculated with uterine material and with colostrum acquired abortion disease.

Although the vaccine injections caused the development of marked agglutination reactions in the treated animals, the reactions in the course of time became much reduced in titer. It was not unusual for the agglutination titers of the principals to increase considerably for a few weeks after the conjunctival exposures and then to subside before parturition.

DISCUSSION OF RESULTS

The results of the experiments were regarded as encouraging. If an attempt had not been made to obtain information as to the possibilities of the intradermic method of vaccine administration, the final results in all probability would have been even more favorable because implantation of the vaccine in the udder seemed to occur more regularly after intradermic than after subcutaneous injections. Moreover, strain 484 was plainly too virulent for use in vaccine, for the three cows which received, either intradermically or subcutaneously, vaccine prepared from this strain, became long-time carriers of the infection in their milk.

None of the *Brucella abortus* strains used for the preparation of vaccine in these experiments were regarded as avirulent. Although the vaccine prepared from strain 11, the least virulent one employed, produced no visible lesions, in the 17 guinea pigs into which it was injected either subcutaneously or intra-abdominally, strain 11 on other occasions caused moderately marked lesions in guinea pigs that were killed two months after injection. Pregnant, susceptible cows that received subcutaneous administrations of vaccine prepared from this strain have, in some cases, maintained serological reactions of such intensity as to indicate localization of the infection in their udders. As the experiments proceeded and further guinea-pig inoculation tests were made of strains 11 and 19, some doubt arose as to whether there was a considerable degree of difference between the virulence of these two strains, the immunizing results of which appeared to be very much alike in cattle.

The results of the experiments failed to indicate that the three *Brucella abortus* strains used in the preparation of the vaccines differed to any marked degree in immunizing effect. From the standpoint of safety, however, it was plainly evident that they differed markedly. Vaccine prepared from as virulent a strain as No. 484 could reasonably be expected to become implanted in the udder of many cows, even if the vaccine were subcutaneously administered when the cows were nonpregnant. Consequently the use of such a strain would be highly objectionable. That strain 19 was more virulent than strain 11 for cattle was evidenced by the fact that the intradermic injection of the former into a nonpregnant cow caused her to eliminate the microorganism in her milk for a prolonged period, whereas after the administration of the latter in the same manner, the infection could not with certainty be detected in the milk at any time. This conclusion was based on the development of moderately marked agglutination reactions of guinea pigs after they were inoculated with milk rather than on the development of abortion disease lesions in these animals. Vaccines prepared from strains 11 and 19, when administered subcutaneously, gave evidence of being noninfective for the udders of nonpregnant cows.

The degree of *Brucella abortus* exposure to which the animals in this experiment were subjected was severe, as is shown by the fact that seven of eight controls had agglutination reactions with titers of 1 to 100 or higher in from 9 to 18 days after exposure. The single exception required a 2-month interval to react with a titer of 1 to 200 but with a titer of 1 to 50 she reacted at the end of 18 days.

No appreciable difference was observed in the immunizing results produced by a 10 c c and a 20 c c dose of abortion vaccine of a density 10 times that of tube 1 of the McFarland nephelometer, when the doses were administered subcutaneously. It seems reasonable to believe that, with a suspension of the density employed, the amount injected could be subjected to even much greater variation without observable differences in results.

The intensity of the thermic reactions that were occasioned by the vaccine injections did not appear to furnish any reliable information as to the degree of immunity which the vaccine might be expected to confer; in fact, the variation in such reactions shown by different animals was slight.

CONCLUSIONS

The data which the experiment yielded seem to justify the following conclusions:

In the preparation of abortion vaccine for use in nonpregnant cattle, the virulence of the *Brucella abortus* strain or strains from which the vaccine is prepared is a matter of great importance, particularly when the vaccine is to be used as an immunizing agent for nonpregnant cows with functioning udders.

The virulence of *Brucella abortus* strains for cattle can be determined with a considerable degree of accuracy by their effects on guinea pigs.

Abortion vaccine prepared from strains of *Brucella abortus* sufficiently virulent to cause extensive lesions in guinea pigs is likely to implant the infection in the udders of nonpregnant cows and may be eliminated in the milk for long periods.

Abortion vaccine prepared from strains of *Brucella abortus* of greatly reduced virulence, as indicated by guinea-pig-inoculation results, gives evidence of being reasonably efficient as an immunizing agent for abortion-disease-free cows and heifers when the vaccine is administered subcutaneously two months or more before service. The danger of such a vaccine being implanted in the udder of the vaccinated animal seems to be slight.

There appears to be more danger of causing infection of the udder, in which it may persist for a long time, if the vaccine is injected intradermically than if it is injected subcutaneously; hence, the former seems to be a less satisfactory method of administration. The conjunctival method of exposure regularly transmits abortion disease to cattle.

EFFICACY OF AN AVIRULENT STRAIN OF BRUCELLA ABORTUS FOR VACCINATING PREGNANT CATTLE [1]

By W. E. COTTON, *Superintendent;* J. M. BUCK, *Assistant Superintendent;* and H. E. SMITH, *Associate Veterinarian, Experiment Station, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

The experiment here discussed is one of a series conducted to develop, if possible, improved means of combating infectious abortion in cattle. This paper, dealing with the vaccination phase of the study, reports a continuation of investigations conducted in 1927 and 1928, the results of which were presented briefly in 1928 [2] and in more detail in 1932.[3] The present experiment makes use of a different method of exposure and a larger number of animals.

Previous work at the Experiment Station,[4] Bethesda, Md., showed that a strain of *Brucella abortus* that had become so attenuated, through long cultivation, that it would no longer produce lesions in guinea pigs unless administered in very large doses, would confer a considerable degree of resistance against virulent strains. The use of a similar strain by Huddleson [5] not only induced immunity in guinea pigs, but also afforded a considerable degree of protection to cattle. Furthermore, Huddleson found that the strain he employed could be given to pregnant cows and heifers with impunity.

Although the investigations of Huddleson led him to conclude that the particular avirulent strain with which he worked increased resistance whether given before or after breeding, the results obtained at the Experiment Station indicated that the avirulent strain there used did not induce sufficient immunity, when given before conception, to protect the animals against a severe exposure, but if administered after conception it offered some protection.

The need for a dependable method of treating pregnant, noninfected animals in infected herds and the encouragement afforded by the results of Huddleson and by those obtained at the Experiment Station prompted further investigation of this phase of the immunization problem.

VACCINE USED AND TESTS FOR VIRULENCE

The *Brucella abortus* strain used in this experiment, designated *Br. abortus* strain 801, was isolated in 1915 from the milk of an infected cow. When the cattle in the experiment were vaccinated, this strain had undergone from 182 to 193 transfers. Despite the fact that it had lived so long on culture media and had given indica-

[1] Received for publication May 26, 1932; issued March, 1933.

[2] MOHLER, J. R. Rpt. of the Chief of the Bur. Anim. Indus., U. S. Dept. Agr. Ann. Rpt., 20-22. 1928.

[3] COTTON, W. E. EFFICACY OF DIFFERENT STRAINS OF BRUCELLA ABORTUS AS IMMUNIZING AGENTS AGAINST INFECTIOUS ABORTION. Jour. Agr. Research 45: 705-724. 1932.

[4] SCHROEDER, E. C., and COTTON, W. E. RECENT BUREAU OF ANIMAL INDUSTRY EXPERIMENT STATION BOVINE INFECTIOUS ABORTION STUDIES. Jour. Amer. Vet. Med. Assoc. 66: 550-561. 1925.

[5] HUDDLESON, I. F. THE VACCINAL IMMUNIZATION OF ANIMALS AGAINST BACTERIUM ABORTUS (BANG) INFECTION. U. S. Livestock Sanit. Assoc. Proc. (1925) 29: 210-215. 1926.

tion of being nonlesion producing for guinea pigs and nonpathogenic for cattle, its agglutinating value had never seemed to be impaired.

To prove its lack of virulence for guinea pigs, groups of these animals were inoculated at irregular intervals from August 17, 1928, to October 14, 1929, with physiological salt solution suspensions of the microorganisms that had been transferred on culture media from 162 to 193 times. Of the 91 guinea pigs used, 73 were injected subcutaneously and 18 intra-abdominally. The suspensions used in making the inoculations had a density equal to 10 times that of tube 1 of the McFarland nephelometer; the subcutaneous dose was ¼ c c and the intra-abdominal dose was 1½ c c.

Six of the guinea pigs were killed and autopsied at the end of six weeks, but the remainder were allowed to live about two months after they were inoculated. No lesions were observed, on autopsy, in any of the guinea pigs except testicle adhesions in six of those that were inoculated intra-abdominally. These lesions were not considered as being characteristic of abortion disease but rather as the result of a large amount of foreign material injected into the peritoneal cavity.

Brucella abortus was isolated from the spleens of 2 of the 6 guinea pigs that were killed at the end of six weeks after inoculation, though these organs showed no abortion-disease lesions. It was not possible, however, to isolate this microorganism from the spleens of 12 other guinea pigs which were permitted to live two months after inoculation.

The avirulent *Brucella abortus* suspensions, though incapable of causing lesions to develop in any of the 91 guinea pigs, nevertheless induced the formation of agglutinins in the blood of all but a small proportion of them. At the time of autopsy a blood sample was taken from each guinea pig. These samples on being tested for *Br. abortus* agglutinins gave the following results:

11 negative in all dilutions, 1–25 to 1–500
7 positive in dilutions of 1–25
15 positive in dilutions of 1–50
17 positive in dilutions of 1–100
19 positive in dilutions of 1–200
22 positive in dilutions of 1–500 or more

The variations in reactions were not confined to any particular group of guinea pigs.

One nonreacting heifer, about five months pregnant, was subjected to an intravenous injection of strain 801 as a preliminary test of its virulence on cattle. This heifer, No. 1336, was bred September 4, 1928. On February 4, 1929, 10 c c of a suspension of strain 801, about twice the density of tube 1 of the McFarland nephelometer, was introduced into one of her jugular veins. Serum agar slants that were sown with a loopful of the material used for the intravenous exposure developed a heavy growth of *Brucella abortus*. Blood serum drawn from the heifer on Febuary 4, just before the intravenous injection, gave negative results to the agglutination test. An agglutination test of her blood serum made March 23, about seven weeks after the injection, showed a titer of 1 to 500. On May 14 the titer had decreased to 1 to 200. The heifer gave birth to a vigorous calf June 17, 1929, 286 days after breeding. Her blood serum then showed slight agglutination in a 1 to 100 dilution.

Her placenta came away promptly. Six guinea pigs which were inoculated with an emulsion of cotyledons, and six guinea pigs which were inoculated with a composite sample of colostrum, were free from evidence of abortion disease when they were killed two months later. Although but one bovine animal was used at this time for determining the virulence of the strain on cattle, the test was considered as being a severe one in view of the fact that the intravenous method was used in introducing the infection.

EXPERIMENT ANIMALS AND METHODS OF VACCINATION

To determine the value of this avirulent strain, administered after conception, in inducing resistance to *Brucella abortus*, use was made of 19 pregnant cows and heifers. Of these 10 were principals and 9 were controls. Five principals, cows 1503 and 1506 and heifers 1497, 1527, and 1534, were vaccinated April 23, 1929; two principals, cow 1494 and heifer 1424, were vaccinated May 11, 1929; and three principals, heifers 1547, 1549, and 1556, were vaccinated October 1, 1929, and again on October 15, 1929.

A large number of the animals used in the experiment were pregnant when purchased, hence no information was available as to their service dates. They were subjected to the tube agglutination test for infectious abortion at the time of purchase. The results were negative in all cases. Examinations of the animals made a few days before they were vaccinated indicated that they were between two and four months pregnant.

The vaccine administered to the five principals on April 23, 1929, was prepared from the one hundred and eighty-second transfer of strain 801. The vaccine administered to the two principals on May 11, 1929, was prepared from the one hundred and eighty-seventh transfer of the strain, and the vaccine administered to the three animals on October 1, 1929, and October 15, 1929, was prepared from the one hundred and ninety-second and one hundred and ninety-third transfers.

The vaccine in all cases was adjusted to a density of about 10 times that of tube 1 of the McFarland nephelometer. Of the animals which were vaccinated but once, four received a 20 c c dose and three received a 10 c c dose. The three animals vaccinated twice received 10 c c of the vaccine on each occasion. When a 20 c c dose was used, half the quantity was injected under the skin in front of each shoulder, but when a 10 c c dose was used, the material was introduced at only one point.

The temperatures of all the vaccinated animals were taken just before the vaccine was injected and daily thereafter for several days. The vaccine injections caused a rise in temperature of 3° to 4° by the second or third day, but by the fourth or fifth day the temperature had usually become normal again. Swellings which appeared at the points of inoculation sometimes reached a diameter of 3 to 4 inches and a thickness of from one-half to three-fourths of an inch by the second or third day, but within a week after the injection they had practically disappeared.

TESTS FOR BRUCELLA ABORTUS IN MILK OF VACCINATED ANIMALS

Samples of milk were obtained from cows 1503, 1506, and 1494 a week after vaccination and at weekly intervals thereafter until nine samples had been obtained from each animal. Four guinea pigs were then inoculated intra-abdominally with 5 c c of a composite sample to determine, if possible, whether *Brucella abortus* had invaded the udders of the cows. Although the development of abortion-disease lesions could hardly be anticipated, even if abortion micro-organisms were present in the milk, it seemed reasonable to expect that if present they would cause reactions in the blood serum of the guinea pigs. In no instance, however, were the results of the blood-serum agglutination tests found to be positive when the guinea pigs were killed about two months after inoculation, nor could *Br. abortus* lesions be detected in the guinea pigs at autopsy.

EXPOSURE OF EXPERIMENT ANIMALS TO BRUCELLA ABORTUS

The vaccinated cattle were exposed to virulent strains of *Brucella abortus* within one to two months after vaccination. Seven principals and seven controls were exposed June 26, 1929, and three principals and two controls were exposed November 18, 1929.

At the time of the earlier exposure, it was not realized that the conjunctival method of exposure regularly transmits abortion disease to cattle, consequently both the conjunctival and ingestion methods were used. However, when the second lot of animals were exposed only the conjunctival method was used. Suspensions of *Br. abortus* were employed for the exposures in all cases. The suspensions used on June 26, 1929, were prepared from two virulent strains of *Br. abortus*, Nos. 484 and 1420. Both represented the sixth transfer. For the conjunctival exposure, a suspension twenty times the density of tube 1 of the McFarland nephelometer was used. From three to four drops were deposited on the conjuctiva of one eye of each animal. For the ingestion exposure, each animal received 100 c c of a suspension, the density of which was about twice that of the McFarland nephelometer tube 1. The three principals and two controls which were exposed on November 18, 1929, received a suspension prepared from the second, fourth, and sixth transfers, respectively, of three virulent *Br. abortus* strains, Nos. 1497, 1456, and 1531. Four drops of the suspension, which was about twenty times the density of the McFarland nephelometer tube 1, were deposited on the conjuctiva of one eye of each animal.

When an animal calved or aborted, six guinea pigs were inoculated with uterine discharges or placental material. Six guinea pigs were also inoculated with 5 c c of a composite sample of colostrum. When animals aborted, cultural studies were made of the fetuses. Table 1 shows the immunizing results of the 19 animals used in the experiment.

TABLE 1.—*Immunizing results obtained by the use of Brucella abortus 801 strain vaccine in 10 principals as compared with the results obtained with 9 controls*

ANIMALS VACCINATED WITH BRUCELLA ABORTUS 801 STRAIN VACCINE

Animal No.	Date of vaccination	Vaccine dose	Date of exposure	Date of calving or aborting	Outcome of pregnancy	Results of uterine examinations for *Br. abortus*[a]	Results of colostrum examinations for *Br. abortus*[a]
		c c					
Cow:							
1503	Apr. 23, 1929	20	June 26, 1929	Sept. 29, 1929	Vigorous calf	−	−
Heifer:							
1497	do	20	do	Aug. 19, 1929	Aborted	+	+
Cow:							
1506	do	20	do	Sept. 4, 1929	Weak calf	+	+
Heifer:							
1527	do	10	do	Dec. 6, 1929	Vigorous calf	−	−
1534	do	10	do	Aug. 10, 1929	Aborted	+	+
Cow:							
1494	May 11, 1929	20	do	Nov. 9, 1929	Vigorous calf	−	−
Heifer:							
1424	do	10	do	Oct. 9, 1929	Weak calf	+	+
1547	Oct. 1, 1929 Oct. 15, 1929	10 10	Nov. 18, 1929	Feb. 24, 1930	Vigorous calf	−	−
1549	Oct. 1, 1929 Oct. 15, 1929	10 10	do	Jan. 27, 1930	Aborted	+	+
1550	Oct. 1, 1929 Oct. 15, 1929	10 10	do	Mar. 13, 1930	Weak calf (died)	+	+

CONTROL ANIMALS NOT VACCINATED

Cow:							
1376			June 26, 1929	July 26, 1929	Aborted	+	+
Heifer:							
1450			do	Nov. 21, 1929	Vigorous calf	−	−
1538			do	Aug. 25, 1929	Aborted	+	+
1540			do	Oct. 26, 1929	do	+	+
Cow:							
1488			do	Aug. 28, 1929	do	+	+
Heifer:							
1531			do	Sept. 17, 1929	do	+	+
1456			do	Sept. 30, 1929	do	+	−
1553			Nov. 18, 1929	Feb. 21, 1930	do	+	+
1554			do	Jan. 5, 1930	Weak calf (died)	+	+

[a] Key: + indicates presence of *Brucella abortus*; − indicates absence of *Br. abortus*.

Of the 10 vaccinated animals which were exposed to virulent strains of *Brucella abortus*, 4 produced vigorous calves. The presence of *Br. abortus* in the uterus or colostrum at the time of calving could not be demonstrated. Three principals produced weak calves, one of which died shortly after birth. *Br. abortus* was found in the uterus and colostrum of each of these cows. Three principals aborted, and *Br. abortus* was isolated from the uterus and colostrum of each of them.

Of the nine control animals only one produced a vigorous calf. Her uterus and colostrum appeared to be free from *Brucella abortus* infection. One control animal produced a calf which did not have sufficient vigor to live. *Br. abortus* infection was demonstrated in the uterus and colostrum of the dam. Seven of the control animals aborted. *Br. abortus* infection was found in the uteri of all seven animals and in the colostrum of six.

In the group of 10 vaccinated animals the placenta was promptly expelled by 8 animals and retained by 2. In the group of 9 control animals the placenta was expelled by 3 animals and retained by 6.

Table 2 gives in detail the records of the 19 animals used in the experiment.

TABLE 2.—*Records of individual experimental animals* [a]

VACCINATED COW 1503

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used [b]						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection 20 c c abortion vaccine.
Apr. 23, 1929	−	−	−	−	−	−	
Apr. 30, 1929	+	+	+	+	P	P	Apr. 30, 1929, milk negative for *Br. abortus*.
May 7, 1929	+	+	+	+	+	P	May 7, 1929, milk negative for *Br. abortus*.
May 14, 1929	+	+	+	+	S	−	May 14, 1929, milk negative for *Br. abortus*.
May 21, 1929	+	+	+	+	+	−	May 21, 1929, milk negative for *Br. abortus*.
June 4, 1929	+	+	+	+	−	−	May 28, 1929, milk negative for *Br. abortus*.
June 11, 1929	+	+	+	+	−	−	June 4, 1929, milk negative for *Br. abortus*.
June 18, 1929	+	+	+	+	−	−	June 11, 1929, milk negative for *Br. abortus*.
June 27, 1929	+	+	P	−	−	−	June 18, 1929, milk negative for *Br. abortus*.
July 2, 1929	+	+	+	P	−	−	June 26, 1929, received *Br. abortus*, conjunctival and ingestion exposure.
July 17, 1929	+	+	+	+	+	−	
July 31, 1929	+	+	+	+	P	−	July 2, 1929, milk negative for *Br. abortus*.
Aug. 27, 1929	+	+	+	S	−	−	Sept. 21, 1929, milk negative for *Br. abortus*.
Sept. 11, 1929	+	+	+	−	−	−	Sept. 29, 1929, produced a vigorous calf; guinea pigs inoculated with uterine material and colostrum failed to acquire abortion disease.
Oct. 8, 1929	+	+	+	−	−	−	
Oct. 23, 1929	+	P	−	−	−	−	Oct. 15, 1929, milk negative for *Br. abortus*.
Nov. 6, 1929	+	+	−	−	−	−	
Nov. 19, 1929	+	P	−	−	−	−	
Dec. 19, 1929	+	P	−	−	−	−	
Jan. 8, 1930	+	P	−	−	−	−	
Jan. 22, 1930	+	S	−	−	−	−	
Feb. 5, 1930	+	+	−	−	−	−	
Mar. 5, 1930	+	+	P	−	−	−	
Apr. 2, 1930	+	S	−	−	−	−	
July 16, 1930	+	P	S	−	−	−	
Oct. 2, 1930	+	+	P	−	−	−	
Nov. 11, 1930	+	P	S	−	−	−	

VACCINATED HEIFER 1497

Apr. 10, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine.
Apr. 23, 1929	−	−	−	−	−	−	
Apr. 30, 1929	+	+	+	+	+	+	
May 7, 1929	+	+	+	+	+	+	
May 14, 1929	+	+	+	+	+	P	
May 21, 1929	+	+	+	+	+	−	
June 4, 1929	+	+	+	+	P	−	
June 11, 1929	+	+	+	+	−	−	
June 18, 1929	+	+	+	+	−	−	
June 27, 1929	+	+	+	P	−	−	June 26, 1929, received *Br. abortus*, conjunctival and ingestion exposure.
July 2, 1929	+	+	+	+	−	−	
July 17, 1929	+	+	+	+	−	−	
July 31, 1929	+	+	+	+	+	+	Aug. 19, 1929, aborted; *Br. abortus* isolated from fetus by cultural methods; guinea pigs inoculated with uterine material and with colostrum developed abortion disease.
Aug. 27, 1929	+	+	+	+	+	+	
Sept. 11, 1929	+	+	+	+	+	+	
Sept. 24, 1929	+	+	+	+	+	+	
Oct. 8, 1929	+	+	+	+	+	+	
Oct. 23, 1929	+	+	+	+	+	+	Oct. 15, 1929, milk positive for *Br. abortus*.
Nov. 6, 1929	+	+	+	+	+	+	

VACCINATED COW 1506

Apr. 10, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine.
Apr. 23, 1929	−	−	−	−	−	−	
Apr. 30, 1929	+	+	+	+	+	+	Apr. 30, 1929, milk negative for *Br. abortus*.
May 7, 1929	+	+	+	+	+	P	May 7, 1929, milk negative for *Br. abortus*.
May 14, 1929	+	+	+	+	+	+	May 14, 1929, milk negative for *Br. abortus*.
May 21, 1929	+	+	+	P	−	−	May 21, 1929, milk negative for *Br. abortus*.
June 4, 1929	+	+	+	+	−	−	May 28, 1929, milk negative for *Br. abortus*.
June 11, 1929	+	+	+	+	−	−	June 4, 1929, milk negative for *Br. abortus*.
June 18, 1929	+	+	+	+	−	−	June 11, 1929, milk negative for *Br. abortus*.
June 27, 1929	+	+	+	P	−	−	June 18, 1929, milk negative for *Br. abortus*.
July 2, 1929	+	+	+	P	−	−	June 26, 1929, received *Br. abortus*, conjunctival and ingestion exposure.
July 31, 1929	+	+	+	+	+	+	

[a] Key: + indicates pronounced clumping of bacteria; P, partial clumping; S, a trace of agglutination; −, no evidence of clumping.

[b] Figures at head of columns indicate cubic centimeters of blood serum. They represent approximate dilutions of 1 to 25, 1 to 50, 1 to 100, 1 to 200, 1 to 500, and 1 to 1,000, respectively, with the amount of antigen (1 c c) used.

TABLE 2.—*Records of individual experimental animals*—Continued

VACCINATED COW 1506—Continued

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Aug. 27, 1929	+	+	+	+	S	−	July 2, 1929, milk negative for *Br. abortus*.
Sept. 11, 1929	+	+	+	+	+	+	Sept. 4, 1929, produced a weak calf; guinea pigs inoculated with uterine material and with colostrum acquired abortion disease.
Sept. 24, 1929	+	+	+	+	+	+	
Oct. 8, 1929	+	+	+	+	+	+	
Oct. 23, 1929	+	+	+	+	+	P	Sept. 24, 1929, milk negative for *Br. abortus*.
Nov. 6, 1929	+	+	+	+	+	+	Oct. 15, 1929, milk negative for *Br. abortus*.
Nov. 19, 1929	+	+	+	+	P	−	
Dec. 19, 1929	+	+	+	+	−	−	
Jan. 8, 1930	+	+	+	+	−	−	
Jan. 22, 1930	+	+	+	P	−	−	
Feb. 5, 1930	+	+	+	P	−	−	
Mar. 5, 1930	+	+	+	−	−	−	
Apr. 2, 1930	+	+	+	−	−	−	
June 16, 1930	+	+	+	P	−	−	Jan. 19, 1931, milk negative for *Br. abortus*.

VACCINATED HEIFER 1527

Apr. 10, 1929	−	−	−	−	−	−	
Apr. 23, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine.
Apr. 30, 1929	+	+	+	+	+	+	
May 7, 1929	+	+	+	+	+	+	
May 14, 1929	+	+	+	+	+	+	
May 21, 1929	+	+	+	+	+	−	
June 4, 1929	+	+	+	+	−	−	
June 11, 1929	+	+	+	+	−	−	
June 18, 1929	+	+	+	+	P	−	
June 27, 1929	+	+	+	+	−	−	June 26, 1929, received *Br. abortus*, conjunctival and ingestion exposure.
July 2, 1929	+	+	P	P	−	−	
July 17, 1929	+	+	+	+	−	−	
July 31, 1929	+	+	+	+	−	−	
Aug. 27, 1929	+	+	S	−	−	−	
Sept. 11, 1929	+	+	+	+	−	−	
Oct. 8, 1929	+	+	P	−	−	−	
Oct. 23, 1929	+	+	+	−	−	−	
Nov. 6, 1929	+	+	+	−	−	−	
Nov. 19, 1929	+	+	P	−	−	−	
Dec. 19, 1929	+	+	P	−	−	−	Dec. 6, 1929, produced a vigorous calf; guinea pigs inoculated with uterine material and with colostrum did not contract abortion disease.
Jan. 8, 1930	+	+	−	−	−	−	
Jan. 22, 1930	+	+	P	−	−	−	
Feb. 5, 1930	+	+	+	−	−	−	
Mar. 5, 1930	+	+	−	−	−	−	
Apr. 2, 1930	+	P	−	−	−	−	

VACCINATED HEIFER 1534

Apr. 10, 1929	−	−	−	−	−	−	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine.
Apr. 23, 1929	−	−	−	−	−	−	
Apr. 30, 1929	−	−	−	−	−	−	
May 7, 1929	+	+	+	+	+	+	
May 14, 1929	+	+	+	+	+	−	
May 21, 1929	+	+	+	+	−	−	
June 4, 1929	+	+	+	P	−	−	
June 11, 1929	+	+	+	−	−	−	
June 18, 1929	+	+	P	−	−	−	
June 27, 1929	+	+	−	−	−	−	June 26, 1929, received *Br. abortus*, conjunctival and ingestion exposure.
July 2, 1929	+	+	P	−	−	−	
July 17, 1929	+	+	+	+	P	−	
July 31, 1929	+	+	+	+	−	−	
Aug. 27, 1929	+	+	+	+	+	S	Aug. 10, 1929, aborted; guinea pigs which were inoculated with uterine material and with colostrum contracted abortion disease.
Sept. 11, 1929	+	+	+	+	+	+	
Sept. 24, 1929	+	+	+	+	+	+	
Oct. 8, 1929	+	+	+	+	+	+	
Oct. 23, 1929	+	+	+	+	+	+	
Nov. 6, 1929	+	+	+	P	P	P	
Nov. 19, 1929	+	+	+	+	+	P	
Jan. 22, 1930	+	+	+	+	+	S	Jan. 21, 1930, milk negative for *Br. abortus*.
Feb. 5, 1930	+	+	+	+	−	−	Feb. 15, 1930, milk negative for *Br. abortus*.

TABLE 2.—*Records of individual experimental animals*—Continued

VACCINATED COW 1494

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
May 7, 1929	−	−	−	−	−	−	May 11, 1929, subcutaneous injection of 20 c c abortion vaccine.
May 14, 1929	−	−	−	−	−	−	
May 21, 1929	+	+	+	+	+	+	May 21, 1929, milk negative for *Br. abortus*.
June 4, 1929	+	+	+	+	+	+	May 28, 1929, milk negative for *Br. abortus*.
June 11, 1929	+	+	+	+	+	+	June 4, 1929, milk negative for *Br. abortus*.
June 18, 1929	+	+	+	+	+	+	June 11, 1929, milk negative for *Br. abortus*.
June 27, 1929	+	+	+	+	+	+	June 18, 1929, milk negative for *Br. abortus*.
July 2, 1929	+	+	+	+	+	+	June 26, 1929, received *Br. abortus*, conjunctival and ingestion exposure.
July 17, 1929	+	+	+	+	+	−	
July 31, 1929	+	+	+	+	+	P	July 2, 1929, milk negative for *Br. abortus*.
Aug. 27, 1929	+	+	P	S	−	−	Aug. 13, 1929, milk negative for *Br. abortus*.
Sept. 11, 1929	+	+	+	+	−	−	Sept. 21, 1929, milk negative for *Br. abortus*.
Sept. 24, 1929	+	+	+	P	−	−	Nov. 9, 1929, produced a vigorous calf; guinea pigs inoculated with uterine material and with colostrum did not contract abortion disease.
Oct. 8, 1929	+	+	+	P	−	−	
Oct. 23, 1929	+	+	P	−	−	−	
Nov. 6, 1929	+	+	P	−	−	−	Aug. 19, 1930, milk negative for *Br. abortus*.
Nov. 19, 1929	+	+	+	P	−	−	

VACCINATED HEIFER 1424

May 7, 1929	−	−	−	−	−	−	May 11, 1929, subcutaneous injection of 10 c c abortion vaccine.
May 14, 1929	−	−	−	−	−	−	
May 21, 1929	+	+	+	+	+	+	
June 4, 1929	+	+	+	+	P	S	
June 11, 1929	+	+	+	+	+	−	
June 18, 1929	+	+	+	+	+	−	
June 27, 1929	+	+	+	P	−	−	June 26, 1929, received *Br. abortus*, conjunctival and ingestion exposure.
July 2, 1929	+	+	+	+	−	−	
July 17, 1929	+	+	+	+	−	−	
July 31, 1929	+	+	+	+	−	−	
Aug. 27, 1929	+	+	+	+	+	P	
Sept. 11, 1929	+	+	+	+	+	+	
Sept. 24, 1929	+	+	+	+	+	+	
Oct. 8, 1929	+	+	+	+	+	+	Oct. 9, 1929, produced a weak calf; guinea pigs inoculated with uterine material and with colostrum contracted abortion disease.
Oct. 23, 1929	+	+	+	+	+	+	
Nov. 6, 1929	+	+	+	+	+	−	
Nov. 19, 1929	+	+	+	+	+	+	

VACCINATED HEIFER 1547

Sept. 11, 1929	−	−	−	−	−	−	Oct. 1, 1929, subcutaneous injection of 10 c c abortion vaccine.
Sept. 24, 1929	−	−	−	−	−	−	
Oct. 8, 1929	+	+	+	+	+	+	Oct. 15, 1929, subcutaneous injection of 10 c c abortion vaccine.
Oct. 23, 1929	+	+	+	+	+	S	
Nov. 6, 1929	+	+	+	+	+	−	Nov. 18, 1929, received *Br. abortus*, conjunctival exposure.
Nov. 19, 1929	+	+	+	+	P	−	
Dec. 19, 1929	+	+	+	P	−	−	Feb. 24, 1930, produced a vigorous calf; guinea pigs inoculated with uterine material and with colostrum did not contract abortion disease.
Jan. 8, 1930	+	+	+	−	−	−	
Jan. 22, 1930	+	+	+	−	−	−	
Feb. 5, 1930	+	+	+	S	−	−	
Feb. 19, 1930	+	+	S	−	−	−	
Mar. 5, 1930	+	+	−	−	−	−	
Mar. 19, 1930	+	+	−	−	−	−	
Apr. 2, 1930	+	+	−	−	−	−	
July 16, 1930	+	+	−	−	−	−	

VACCINATED HEIFER 1549

Sept. 11, 1929	−	−	−	−	−	−	Oct. 1, 1929, subcutaneous injection of 10 c c abortion vaccine.
Sept. 24, 1929	−	−	−	−	−	−	
Oct. 8, 1929	+	+	+	+	P	S	Oct. 15, 1929, subcutaneous injection of 10 c c abortion vaccine.
Oct. 23, 1929	+	+	+	+	+	+	
Nov. 6, 1929	+	+	+	+	+	+	Nov. 18, 1929, received *Br. abortus*, conjunctival exposure.
Nov. 19, 1929	+	+	+	+	+	+	
Dec. 19, 1929	+	+	+	+	+	+	Jan. 27, 1930, aborted; *Br. abortus* isolated from both uterine material and colostrum through inoculation of guinea pigs.
Jan. 8, 1930	+	+	+	+	+	+	
Jan. 22, 1930	+	+	+	+	+	+	
Feb. 5, 1930	+	+	+	+	+	+	
Feb. 19, 1930	+	+	+	+	+	+	
Mar. 5, 1930	+	+	+	+	+	+	
Mar. 19, 1930	+	+	+	+	+	+	
Apr. 2, 1930	+	+	+	+	+	+	
May 17, 1930	+	+	+	+	+	+	

TABLE 2.—*Records of individual experimental animals*—Continued

VACCINATED HEIFER 1556

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Sept. 11, 1929	−	−	−	−	−	−	Oct. 1, 1929, subcutaneous injection of 10 c c abortion vaccine.
Sept. 24, 1929	−	−	−	−	−	−	
Oct. 8, 1929	+	+	+	P	S	−	Oct. 15, 1929, subcutaneous injection of 10 c c abortion vaccine.
Oct. 23, 1929	+	+	+	+	+	+	
Nov. 6, 1929	+	+	+	+	+	−	Nov. 18, 1929, received *Br. abortus*, conjunctival exposure.
Nov. 19, 1929	+	+	+	+	−	−	
Dec. 19, 1929	+	+	+	+	+	+	
Jan. 8, 1930	+	+	+	+	+	+	
Jan. 22, 1930	+	+	+	+	+	+	
Feb. 5, 1930	+	+	+	+	+	+	
Feb. 19, 1930	+	+	+	+	S	−	
Mar. 5, 1930	+	+	+	+	−	−	
Mar. 19, 1930	+	+	+	+	+	P	Mar. 13, 1930, produced a weak calf which died; guinea pigs inoculated with uterine material and colostrum contracted abortion disease.
Apr. 2, 1930	+	+	+	+	+	+	
July 16, 1930	+	+	P	S	−	−	
Aug. 19, 1930	+	+	+	+	P	−	Aug. 19, 1930, milk negative for *Br. abortus*.
Oct. 2, 1930	+	+	+	+	+	−	

CONTROL COW 1376

Apr. 10, 1929	−	−	−	−	−	−	June 26, 1929, received *Br. abortus*, conjunctival and ingestion exposure.
May 21, 1929	−	−	−	−	−	−	
June 11, 1929	−	−	−	−	−	−	
June 27, 1929	−	−	−	−	−	−	
July 2, 1929	−	−	−	−	−	−	
July 17, 1929	+	+	+	+	+	−	July 26, 1929, aborted; guinea pigs inoculated with uterine material and with colostrum contracted abortion disease.
July 31, 1929	+	+	+	+	S	−	
Aug. 27, 1929	+	+	+	+	+	−	
Sept. 11, 1929	+	+	+	+	+	+	
Sept. 24, 1929	+	+	+	+	+	+	
Oct. 8, 1929	+	+	+	+	+	+	
Oct. 23, 1929	+	+	+	+	+	+	Oct. 15, 1929, milk positive for *Br. abortus*.
Nov. 6, 1929	+	+	+	+	+	+	
Nov. 19, 1929	+	+	+	+	+	+	
Jan. 22, 1930	+	+	+	+	+	S	Jan. 21, 1930, milk positive for *Br. abortus*.
Feb. 5, 1930	P	P	P	P	−	−	

CONTROL HEIFER 1450

Apr. 10, 1929	−	−	−	−	−	−	
May 21, 1929	P	−	−	−	−	−	
June 11, 1929	S	−	−	−	−	−	
June 27, 1929	−	−	−	−	−	−	June 26, 1929, received *Br. abortus*, conjunctival and ingestion exposure.
July 2, 1929	−	−	−	−	−	−	
July 17, 1929	+	P	−	−	−	−	
July 31, 1929	+	P	−	−	−	−	
Aug. 27, 1929	S	−	−	−	−	−	
Sept. 11, 1929	−	−	−	−	−	−	
Sept. 24, 1929	−	−	−	−	−	−	
Oct. 8, 1929	−	−	−	−	−	−	
Oct. 23, 1929	S	−	−	−	−	−	
Nov. 6, 1929	−	−	−	−	−	−	
Nov. 19, 1929	−	−	−	−	−	−	Nov. 21, 1929, produced a vigorous calf; guinea pigs inoculated with uterine material and with colostrum did not acquire abortion disease.
Jan. 8, 1930	−	−	−	−	−	−	
Jan. 22, 1930	−	−	−	−	−	−	
Feb. 5, 1930	S	−	−	−	−	−	

CONTROL HEIFER 1538

Apr. 10, 1929	−	−	−	−	−	−	
May 21, 1929	−	−	−	−	−	−	
June 11, 1929	−	−	−	−	−	−	
June 27, 1929	−	−	−	−	−	−	June 26, 1929, received *Br. abortus*, conjunctival and ingestion exposure.
July 2, 1929	−	−	−	−	−	−	
July 17, 1929	+	+	+	+	+	+	
July 31, 1929	+	+	+	+	+	+	

TABLE 2.—*Records of individual experimental animals*—Continued

CONTROL HEIFER 1538—Continued

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Aug. 27, 1929	+	+	+	+	+	S	Aug. 25, 1929, aborted; guinea pigs inoculated with uterine material and colostrum contracted abortion disease.
Sept. 11, 1929	+	+	+	+	+	+	
Sept. 24, 1929	+	+	+	+	+	+	
Oct. 8, 1929	+	+	+	+	+	+	
Oct. 23, 1929	+	+	+	+	+	−	Oct. 15, 1929, milk positive for *Br. abortus*.
Nov. 6, 1929	+	+	+	+	+	−	
Nov. 19, 1929	+	+	+	+	+	−	
Jan. 22, 1930	+	+	+	+	+	+	Jan. 21, 1930, milk positive for *Br. abortus*.
Feb. 5, 1930	+	+	+	+	+	+	Feb. 15, 1930, milk positive for *Br. abortus*.

CONTROL HEIFER 1540

Apr. 10, 1929	−	−	−	−	−	−	
May 21, 1929	−	−	−	−	−	−	
June 11, 1929	−	−	−	−	−	−	
June 27, 1929	−	−	−	−	−	−	June 26, 1929, received *Br. abortus*, conjunctival and ingestion exposure.
July 2, 1929	−	−	−	−	−	−	
July 17, 1929	+	+	+	+	−	−	
July 31, 1929	+	+	+	+	−	−	
Aug. 27, 1929	+	+	+	+	+	S	
Sept. 11, 1929	+	+	+	+	+	+	
Sept. 24, 1929	+	+	+	+	+	+	
Oct. 8, 1929	+	+	+	+	+	+	
Oct. 23, 1929	+	+	+	+	+	+	Oct. 26, 1929, aborted; *Br. abortus* isolated from uterine material and colostrum through guinea-pig inoculations.
Nov. 6, 1929	+	+	+	+	+	+	
Nov. 19, 1929	+	+	+	+	+	+	

CONTROL COW 1488

Apr. 10, 1929	−	−	−	−	−	−	June 26, 1929, received *Br. abortus*, conjunctival and ingestion exposure.
May 21, 1929	−	−	−	−	−	−	
June 11, 1929	−	−	−	−	−	−	Aug. 28, 1929, aborted; guinea pigs inoculated with uterine material and colostrum contracted abortion disease.
June 27, 1929	−	−	−	−	−	−	
July 2, 1929	−	−	−	−	−	−	
July 17, 1929	+	+	+	+	+	−	
July 31, 1929	+	+	+	+	+	+	
Aug. 27, 1929	+	+	+	+	+	S	
Sept. 11, 1929	+	+	+	+	+	+	
Sept. 24, 1929	+	+	+	+	+	+	
Oct. 8, 1929	+	+	+	+	+	+	
Oct. 23, 1929	+	+	+	+	+	+	
Nov. 6, 1929	+	+	+	+	+	+	
Nov. 19, 1929	+	+	+	+	+	+	
Jan. 22, 1930	+	+	+	+	+	+	Jan. 21, 1930, milk positive for *Br. abortus*.
Feb. 5, 1930	+	+	+	+	+	+	Feb. 15, 1930, milk positive for *Br. abortus*.

CONTROL HEIFER 1531

Apr. 10, 1929	−	−	−	−	−	−	
May 21, 1929	−	−	−	−	−	−	
June 11, 1929	−	−	−	−	−	−	
June 27, 1929	−	−	−	−	−	−	June 26, 1929, received *Br. abortus*, conjunctival and ingestion exposure.
July 2, 1929	−	−	−	−	−	−	
July 17, 1929	+	+	+	+	−	−	
July 31, 1929	+	+	+	−	−	−	
Aug. 27, 1929	+	+	+	+	S	−	
Sept. 11, 1929	+	+	+	+	+	+	Sept. 17, 1929, aborted; guinea pigs inoculated with uterine material and colostrum contracted abortion disease.
Sept. 24, 1929	+	+	+	+	+	+	
Oct. 8, 1929	+	+	+	+	+	+	
Oct. 23, 1929	+	+	+	+	+	P	Oct. 15, 1929, milk positive for *Br. abortus*.
Nov. 6, 1929	+	+	+	+	P	P	
Nov. 19, 1929	+	+	+	P	P	P	

TABLE 2.—*Records of individual experimental animals*—Continued

CONTROL HEIFER 1456

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929	−	−	−	−	−	−	
May 21, 1929	−	−	−	−	−	−	
June 11, 1929	−	−	−	−	−	−	
June 27, 1929	−	−	−	−	−	−	June 26, 1929, received *Br. abortus*, conjunctival and ingestion exposure.
July 2, 1929	−	−	−	−	−	−	
July 17, 1929	+	+	+	+	+	−	
July 31, 1929	+	+	+	+	−	−	
Aug. 27, 1929	+	+	+	−	−	−	
Sept. 11, 1929	+	+	+	+	+	−	Sept. 30, 1929, aborted; *Br. abortus* isolated from uterus through guinea-pig inoculations, but guinea pigs inoculated with colostrum failed to acquire abortion disease.
Sept. 24, 1929	+	+	+	+	+	+	
Oct. 8, 1929	+	+	+	+	P	−	
Oct. 23, 1929	+	+	+	+	+	+	Oct. 15, 1929, milk negative for *Br. abortus*.
Nov. 6, 1929	+	+	+	+	+	+	
Nov. 19, 1929	+	+	+	+	+	−	
Jan. 22, 1930	+	+	+	+	+	P	Jan. 21, 1930, milk negative for *Br. abortus*.
Feb. 5, 1930	+	+	+	+	P	−	
Apr. 2, 1930	+	+	+	+	−	−	
Apr. 16, 1930	+	+	+	−	−	−	

CONTROL HEIFER 1553

Nov. 6, 1929	−	−	−	−	−	−	
Nov. 19, 1929	−	−	−	−	−	−	Nov. 18, 1929, received *Br. abortus*, conjunctival exposure.
Dec. 19, 1929	+	+	+	+	−	−	
Jan. 8, 1930	+	+	+	+	−	−	
Jan. 22, 1930	+	+	+	+	P	−	
Feb. 5, 1930	+	+	+	+	+	+	
Feb. 19, 1930	+	+	+	+	+	+	Feb. 21, 1930, aborted; guinea pigs inoculated with uterine material and with colostrum contracted abortion disease.
Mar. 5, 1930	+	+	+	+	+	+	
Mar. 19, 1930	+	+	+	+	+	+	
Apr. 2, 1930	+	+	+	+	+	+	
Apr. 16, 1930	+	+	+	+	+	+	
May 7, 1930	+	+	+	+	+	+	
July 16, 1930	+	+	+	+	+	+	Aug. 16, 1930, milk positive for *Br. abortus*.
Aug. 19, 1930	+	+	+	+	+	+	Oct. 21, 1930, milk positive for *Br. abortus*.

CONTROL HEIFER 1554

Nov. 6, 1929	−	−	−	−	−	−	Nov. 18, 1929, received *Br. abortus*, conjunctival exposure.
Nov. 19, 1929	−	−	−	−	−	−	
Dec. 19, 1929	+	+	+	+	−	−	Jan. 5, 1930, produced a weak calf which died; guinea pigs inoculated both with uterine material and with colostrum acquired abortion disease.
Jan. 8, 1930	+	+	+	+	−	−	
Jan. 22, 1930	+	+	+	+	+	+	
Feb. 5, 1930	+	+	+	+	+	+	
Feb. 19, 1930	+	+	+	+	+	+	
Mar. 5, 1930	+	+	+	+	+	+	
Mar. 19, 1930	+	+	+	+	+	+	
Apr. 2, 1930	+	+	+	+	+	+	
Apr. 16, 1930	+	+	+	+	+	+	
May 7, 1930	+	+	+	+	+	+	
July 16, 1930	+	+	+	+	+	+	
Aug. 19, 1930	+	+	+	+	+	+	

DISCUSSION OF RESULTS

The experiment indicates that the use of vaccine prepared from an avirulent *Brucella abortus* strain (No. 801), administered during pregnancy, could not be depended upon to confer a pronounced degree of immunity against abortion disease. On the other hand, the vaccine may be considered as having exerted some beneficial effects, for whereas 40 per cent of the principals withstood the degree of *Br.*

abortus exposure to which they were subjected, only 11 per cent of the control animals resisted it; and whereas 89 per cent of the control animals aborted or produced weak calves, only 60 per cent of the vaccinated animals had similar histories.

The *Brucella abortus* exposure given in the experiment was sufficiently severe to cause all but one of the nine control animals to develop marked reactions to the abortion agglutination test and either to abort or to produce weak calves which did not survive. Agglutination tests were not made with sufficient frequency to determine the exact length of time that elapsed between *Br. abortus* exposure and the appearance of agglutinins in the blood. The tests made, however, showed that six of the nine control animals acquired agglutination titers of from 1 to 100 to 1 to 1,000 in 21 days. Two controls acquired 1 to 200 titers in 31 days. The control that calved normally failed to develop a titer of more than 1 to 50 after exposure to *Br. abortus*. This was the only animal of the 19 used in the experiment which, previous to being subjected to artificial *Br. abortus* exposure, had had a titer of 1 to 25. It is possible that some earlier natural exposure to the disease might have been responsible for the immunity which she manifested in the experiment.

Failure of the vaccinated cattle to resist more generally the exposure to which they were subjected may have been due in part to the fact that a comparatively brief interval, from one to two months, elapsed between the vaccination dates and the dates of exposure to *Brucella abortus*. In view of the nature of the earlier results obtained with this avirulent strain, it seems possible that a considerable period must elapse after vaccination before a serviceable degree of immunity can reasonably be expected. Whether vaccination in abortion disease is followed by a negative phase of resistance and, if so, how long this phase continues, are matters on which few data have been accumulated.

SUMMARY

To determine the efficacy of an avirulent strain of *Brucella abortus* for vaccinating pregnant cattle against abortion disease, vaccines were prepared and administered subcutaneously to three pregnant cows and seven pregnant heifers.

The strain of *Brucella abortus* used for the vaccines was isolated in 1915 from the milk of an infected cow and had undergone from 182 to 193 transfers on artificial culture media when the vaccines were prepared.

Inoculation tests showed the strain, designated as No. 801, to be nonpathogenic for cattle and guinea pigs. The agglutinating value of the strain, however, was not impaired.

From one to two months after vaccination 10 experiment animals were exposed to virulent strains of *Brucella abortus;* 9 other animals were used as controls.

The vaccines gave evidence of conferring immunity against abortion disease to 40 per cent of the vaccinated animals; only 11 per cent of the control animals resisted the disease.

No evidence was obtained to indicate that the strain becomes localized in the udders of vaccinated animals.

BIOLOGY OF THE FLOUR BEETLES, TRIBOLIUM CONFUSUM DUV. AND T. FERRUGINEUM FAB.[1]

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INTRODUCTION

The flour beetles have long been known to be among the more serious pests of flour, meal, and other cereal products. Numerous references in the literature give ample evidence of their importance as destroyers of these foodstuffs. However, owing probably to the rather long adult life of these insects and the difficulties in observing the immature stages, no thorough work has been done on their life history. Several suppositions of early authors regarding the life cycle have been copied and recopied until now these statements, often erroneous, are accepted as facts. The following preliminary account is a summary of rather extensive experiments on these two species commenced by the writer in the latter part of 1929.

DIFFERENTIATING CHARACTERS OF ADULTS

The two species *Tribolium confusum* Duv. and *T. ferrugineum* Fab. are so similar in size, color, shape of body, and habits that they are continually confused, even by experienced entomologists. The distinguishing characters are so constant, however, that once these are learned there can never be any doubt as to their identity. The character found most useful by the writer for distinguishing the two species is the size of the eyes on the ventral surface of the head. In *T. ferrugineum* the width of each eye is approximately equal to the distance separating them on the underside of the head. In *T. confusum* the eyes, viewed from below, appear small, the width of each eye being approximately only one-third that of the distance separating them. This character can be used for the identification of living specimens, whereas the character usually given—i. e., the sudden enlargement of the last three joints of the antennae in *T. ferrugineum* and the gradual enlargement in *T. confusum*—is almost useless for identifying living specimens because the antennae are usually in motion. Another differentiating trait, useful for field identification but rather unreliable, is that when placed on a flat surface under a strong light *T. ferrugineum* often attempts to fly, and sometimes makes short flights of a few feet, while *T. confusum* never makes any attempt at flight.

Careful measurements of 50 adults show them to be somewhat smaller than the measurements usually given. *Tribolium confusum* is found to average 3.47 mm in length and 1.07 mm in width through the prothorax. These measurements are almost identical with those

[1] Received for publication June 6, 1932; issued March, 1933.

given by Brindley, [2] but are considerably smaller than those of Chapman [3] and other authors. *T. ferrugineum* averages 3.32 mm in length and 1.03 mm in width through the prothorax.

ORIGIN, HABITAT, AND DISTRIBUTION

The origin of insects infesting stored products is difficult to determine because they are widely distributed by commerce and most of them have long been cosmopolitan pests. In the case of *Tribolium ferrugineum* a hint as to its probable origin is given by Blair,[4] who refers to it under the name *T. castaneum* Hbst. He shows that in India this species is commonly found in the wild state under bark and in wood. It is also found in such situations in North America and elsewhere, but not at all frequently. It is an insect of subtropical climates, and very likely India is its original home. This view is strengthened by the fact that, in the same article, Blair has described a very closely related species, *T. indicum*, from the same country and with the same habitat, which does not occur in stored products.

As to the original habitat of the genus, there can be no reasonable doubt that before the advent of civilized man it lived under bark and in old logs. Here it was probably a scavenger, but this can not be definitely proved. Almost without exception, the beetles of the subfamily Ulominae, of which Tribolium is a member, occur either as pests of stored products or else under the bark of trees and in rotting logs. It seems evident that all the members of this group originally lived in the latter habitat and have recently adopted the flour-feeding habit. Two species of Tribolium, *T. madens* Charp. and *T. indicum* Blair, are found almost exclusively in such situations, and the two flour pests *T. confusum* and *T. ferrugineum* are themselves occasionally found there.

These two species are known to be cosmopolitan, occurring throughout the world in stored cereal products. Temperature seems to influence their distribution somewhat. *Tribolium ferrugineum* is essentially an insect of warm climates, being seldom taken north of the fortieth parallel. *T. confusum*, on the other hand, seems to prefer cooler climates and is most abundant in the northern part of the United States.

FOOD

A wide variety of foodstuffs are attacked by Tribolium, a list of which is given by Chittenden.[5] Practically any kind of flour, meal, breakfast food, and cracked grain is attacked, and much damage is done to dried insect specimens in collections. The writer has found these pests as much to be feared in insect collections as the dermestids. They are also known to breed in certain spices. Three other preferred foods which seem to have received little or no mention are chocolate, raisins, and various nuts, especially Persian (English) walnuts, on which the larvae develop very rapidly.

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[2] BRINDLEY, T. A. THE GROWTH AND DEVELOPMENT OF EPHESTIA KUEHNIELLA ZELLER (LEPIDOPTERA) AND TRIBOLIUM CONFUSUM DUVAL (COLEOPTERA) UNDER CONTROLLED CONDITIONS OF TEMPERATURE AND RELATIVE HUMIDITY. Ann. Ent. Soc. Amer. 23: 741–757, illus. 1930.

[3] CHAPMAN, R. N. THE CONFUSED FLOUR BEETLE (TRIBOLIUM CONFUSUM DUVAL). Minn. State Ent. Rpt. 17: 73–94, illus. 1918.

[4] BLAIR, K. G. THE INDIAN SPECIES OF PALORUS, MULS, (COLEOPTERA: TENEBRIONIDAE) AND SOME ASSOCIATED BEETLES. Indian Forest Rec. 14 (5): 1–20 (133–152), illus. 1930.

[5] CHITTENDEN, F. H. INSECTS AFFECTING CEREALS AND OTHER DRY VEGETABLE FOODS. Chapter 8, in The Principal Household Insects of the United States. U. S. Dept. Agr., Div. Ent. Bul. (n. s.) 4: 112–131, illus. 1896.

OVERWINTERING

In heated flour mills, warehouses, etc., Tribolium breed the year around and all stages can be found at any time during the year. In unheated flour mills this is not the case. Some authors have stated that these beetles do not live over winter in unheated mills but that these mills are reinfested in the spring from neighboring heated mills. An inspection of several unheated flour mills in Maryland and northern Virginia during February, 1931, revealed many adults of *T. confusum* in a semidormant condition, but no living larvae or pupae. As the adults usually live a year, or over, it is evident that in unheated flour mills in this territory the winter is passed in the adult stage and breeding begins with the approach of spring. In the Gulf States breeding probably continues the year around, while in the extreme Northern States and Canada the species may not be able to survive the winter except in heated buildings. *T. ferrugineum* seems to be less resistant to cold than *T. confusum*.

LONGEVITY

It has been known that Tribolium adults are long-lived, but no actual tests have previously been made to determine the maximum length of life. Estimates have ranged from three months to a year or more. As many of the adults under observation by the writer are still alive, no definite statement can yet be made as to the maximum length of life except that it is considerably longer than is generally supposed. Of 50 individuals of *T. confusum* emerging nearly 24 months previous to this writing (January, 1932), 13 are still alive and active. *T. ferrugineum* appears to be somewhat shorter lived than *T. confusum*, but 5 individuals out of an original 60 are still alive after 23 months.

OVIPOSITION

Of all phases of the life history of Tribolium, oviposition seems to have received the least attention. Only two investigators, Chapman and Brindley, appear to have done any work on the oviposition of these insects, and they have been concerned only with the number of eggs laid per day by young females and have made no attempt to determine the total number of eggs laid or the length of the oviposition period. Inasmuch as the oviposition period may last for more than a year and it is almost impossible to locate the eggs in the flour, it is not surprising that more work has not been done along this line.

In the experiments made by the writer 25 pairs of each species were segregated on emergence and placed in different foods under different conditions of temperature and humidity. Small vials, lightly stoppered with cotton, were used as containers. A single pair of adults was placed in each vial and moved to another vial every day. Various methods of locating the eggs were tried, but it was found that the method, first advocated by Chapman, of counting the larvae rather than the eggs was more accurate. As approximately 90 per cent of the eggs hatched, the actual number of eggs laid is about one-ninth greater than the figures given, which concern viable eggs only.

Table 1 gives the average duration of the egg-laying period and the average number of eggs laid for each group of females used in the experiments.

TABLE 1.—*Summary of data concerning oviposition of Tribolium confusum and T. ferrugineum*

T. CONFUSUM

Temperature	Food	Females	Average oviposition period	Average eggs laid	Average eggs laid per day
		Number	*Days*	*Number*	*Number*
27° C.	Whole-wheat flour	6	214	521	2.43
	Bran	6	263	333	1.26
	Oatmeal	2	136	141	1.04
	White flour	3	325	187	.58
Room	Whole-wheat flour	7	280	744	2.66

T. FERRUGINEUM

27° C.	Whole-wheat flour	6	171	438	2.56
	Middlings	3	102	246	2.41
	Bran	3	147	235	1.60
Room	Whole-wheat flour	3	160	518	3.24
	Corn meal	3	228	500	2.19
	Middlings	2	143	302	2.11
	Oatmeal	3	245	123	.50
	White flour	2	73	19	.26

The longest oviposition period observed was that of a female of *Tribolium confusum* in whole-wheat flour at room temperature. This female laid viable eggs for 432 days. The longest period for *T. ferrugineum* was 308 days, for a female that was kept in oatmeal at room temperature. The greatest number of viable eggs laid was 976, by a female of *T. confusum* also kept in whole-wheat flour at room temperature. The greatest number of viable eggs laid by *T. ferrugineum* was 956; this individual was kept in corn meal at room temperature. The number of eggs laid per day is not large. In no case were more than 13 viable eggs laid in one day by a single female, and the average was only 2 or 3 per day. Under optimum conditions Brindley [6] records 18 eggs in one day and a much higher daily average than is indicated here.

Mating was observed rather frequently among the pairs of adults in the experiment. The writer's records show that the female may continue to lay viable eggs for as long as five months after being separated from the male.

THE EGG STAGE

The eggs are usually laid singly, directly in the flour, but occasionally they are found attached to the sides of the container. They are coated with a sticky substance which causes the flour to adhere to them. The length of the incubation period varies considerably with the external conditions. Fifty eggs of *Tribolium ferrugineum* kept in an incubator at 27° C. hatched in an average period of 5.5 days. Forty eggs kept at room temperature averaged 8.6 days. Here the temperature ranged from 18.5° to 28.5° and averaged 22°, while the humidity ranged from 22 to 43 per cent and averaged 32 per cent.

The incubation period for *Tribolium confusum* is slightly longer. Forty eggs kept in an incubator at 27° C. hatched in an average period of 6.8 days. Of these, 17 kept in continuous light averaged 6.5 days,

[6] Brindley, T. A. Op. cit.

and 23 kept in continuous darkness averaged 7 days. Forty eggs kept at room temperature required on an average 12.8 days to hatch. In this case the temperature ranged from 14.5° to 26° and averaged 21°, while the relative humidity ranged from 24 to 51 per cent and averaged 34 per cent.

THE LARVAL PERIOD

Chapman[7] and Brindley[8] found six larval instars in *Tribolium confusum*. Through continued observation the writer has determined that there is no fixed number of larval molts, but that the number ranges from 6 to 11 or more and is normally 7 or 8 instead of 6. This variation is due both to external conditions, such as food, temperature, and humidity, and to individual characteristics entirely apart from external influences.

The number of larval instars was determined by actual count of the number of times individual larvae molted before pupation. Over 100 eggs of both species of Tribolium were placed in individual containers and observed each day until the individuals emerged as adults. Varying conditions of food, temperature, and humidity were used. After each molt the exuviae could be seen in the small vial used as a container and were immediately removed and recorded.

Table 2 gives a summary of experiments to determine the number and duration of the larval instars under various conditions.

[7] Chapman, R. N. Op. cit. [8] Brindley, T. A. Op. cit.

TABLE 2.—*Summary of data on the larval period of Tribolium confusum and of T. ferrugineum*

Temperature	Food	Larvae	Average duration of each larval instar [a]											Entire larval period
			First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth	Eleventh	
		Number	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*
T. CONFUSUM														
27° C.	Middlings	4	1.6	4.5	4.0	4.2	4.5	4.5	6.5	5.0(1)				31.0
	Bran	11	2.2	5.7	6.5	6.4	6.5	6.6	6.7	7.3(7)	8.0(1)			45.7
	Oatmeal	5	2.0	5.8	6.8	8.2	8.0	13.2	8.4	9.4	8.0(1)	7.0(1)		64.6
	White flour	8	2.0	4.9	7.5	7.5	12.0	16.5	14.2(5)	19.7(3)				89.0(3)
T. FERRUGINEUM														
27° C.	Bran	7	2.0	4.4	4.0	4.1	4.1	6.1	7.0(4)					28.9
	Whole-wheat flour	7	1.7	4.3	4.0	4.1	4.3	4.9	6.1	7.0(1)				30.4
	Corn meal	8	2.0	4.6	5.0	4.0	4.5	6.1	7.6(5)	7.0(1)				31.9
	Middlings	7	2.0	4.7	4.3	4.0	4.3	6.0	7.0(4)	7.3(3)				32.6
	Oatmeal	7	1.9	5.6	7.9	8.9	10.6	11.1	9.5(6)	10.7(6)	8.5(2)	9.0(1)		68.2(4)
	White flour	7	2.0	5.6	5.9	9.4	18.0	21.7(3)	17.0(1)	26.0(1)	14.0(1)			87.5(2)
Room	Middlings	7	2.3	7.9	6.0	4.9	4.6	7.6	12.7(3)	14.0(1)				40.4
	Whole-wheat flour	6	2.2	8.3	6.5	4.8	4.5	7.2	11.0(5)	13.0(2)				46.8
	Bran	4	2.7	9.2	6.5	5.3	5.3	6.0	12.5	6.0(1)	11.0(1)			51.5
	Corn meal	7	2.6	10.6	8.3	6.3	7.0	8.9	10.1	10.7(6)	9.0(1)			64.9
	White flour	4	2.3	8.5	9.0	14.2	21.5	20.5	14.0	21.0(2)	20.0(1)			79.8(3)
	Oatmeal	3	2.7	10.0	12.0	11.3	12.7	12.0	8.3	8.0	9.7	11.7	15.7	114.0

[a] Numbers in parenthesis indicate number of individuals passing through any molt if less than original number.

There is considerable variation in the length of the larval period due both to the kind of food and to the temperature. Whole wheat flour, middlings, bran, and corn meal are all acceptable foods. Growth is very slow in white flour and many of the larvae die before reaching the adult stage. Under certain conditions Persian walnuts have been found to be very favorable for larval development, some of the shortest life cycles being recorded with this food. A constant temperature of 27° C. proved to be much more suitable for development than ordinary room temperatures. In Table 2 the room temperatures were those found in the laboratory at Washington, D. C., during April and May.

There is considerable individual variation but practically no overlapping of the measurements of an individual of one instar with those of the next. The following tabulation shows the average widths of the head capsules of 40 larvae of *Tribolium confusum* of the different instars:

Instar	Width (mm) of head capsule	Instar	Width (mm) of head capsule
First	0. 175	Sixth	0. 459
Second	. 197	Seventh	. 585
Third	. 249	Eighth	. 619
Fourth	. 311	Ninth	. 655
Fifth	. 387		

THE PUPAL PERIOD

When ready to pupate, the mature larva comes to the surface of the food in which it has been working and, after a short prepupal period, transforms to the naked pupa, lying in or on the surface of the food without protection of any kind. The vacated pupal cells of the Mediterranean flour moth are often found to contain several Tribolium pupae.

The average duration of the pupal period was found to be as follows:

Thirty-one *T. confusum* pupae kept at 27° C. in continuous darkness emerged as adults in from 7 to 12 days, with an average of 8.74 days.

Forty-three *T. confusum* pupae kept at 27° C. in continuous light emerged as adults in from 6 to 9 days, with an average of 7.86 days.

One hundred and twelve *T. ferrugineum* pupae kept at 27° C., for the most part in continuous darkness, had a pupal period of from 6 to 9 days, with an average of 7.14 days.

Thirty-two *T. ferrugineum* pupae kept at room temperature during the early summer had a pupal period of from 5 to 14 days, with an average of 8.5 days.

It is during the pupal period only that the sexes can be distinguished. Chapman [9] gives a good illustration of the differences in the terminal segment of the abdomen. On the female there is a pair of appendages in addition to the regular terminal cerci, while the terminal segment of the male appears to have only a disklike depression on this segment.

PARASITES

The flour beetles seem to be comparatively free from parasitic enemies. Two mites, *Acarophenax tribolii* Newstead and Duval and *Pediculoides ventricosus* Newport, and a bethylid, *Rhabdepyris zeae* Waterston, have been recorded as attacking Tribolium, but none seem to be very effective in controlling these pests.

[9] Chapman, R. N. Op. cit.

SUMMARY

The flour beetles *Tribolium confusum* Duv. and *T. ferrugineum* Fab. are among the more serious pests of flour and other cereal products. They attack practically any kind of flour, meal, breakfast food, or cracked grain, many kinds of spices, and various nuts, chocolate, and raisins. They are also serious pests in insect collections.

The size of the eyes on the ventral surface of the head is the best character for distinguishing the two species.

The winter is passed in the adult state in the central and northern parts of the United States. Adults of both species may live two years or even longer.

The oviposition period of *T. confusum* may last as long as 14 months, the average being about 9 months. That of *T. ferrugineum* is slightly less. A female of either species usually lays 400 to 500 eggs during this time; in some cases nearly 1,000 eggs have been laid by a single female.

The average incubation period at 27° C. is 6.8 days for *T. confusum* and 5.5 days for *T. ferrugineum*.

The number of larval instars ranges from 6 to 11, with an average of 7 or 8. The larval period at 27° C. ranges from 27 to 90 days according to the food. It is slightly longer for *T. confusum* than for *T. ferrugineum*. Whole-wheat flour, middlings, bran, corn meal, oatmeal, and white flour, arranged in the order of their acceptability, are the foods used in the experiments. Measurements of the head capsule in larvae of *T. confusum* are given.

The pupal period at 27° C. averages 8.2 days for *T. confusum* and 7.1 days for *T. ferrugineum*. Lower temperatures lengthened all stages considerably.

Enemies of the flour beetles include two mites and a bethylid.

HYPOSOTER DISPARIS VIERECK, AN INTRODUCED ICHNEUMONID PARASITE OF THE GIPSY MOTH [1]

By C. F. W. MUESEBECK, *Senior Entomologist*, and D. L. PARKER, *Junior Entomologist, Division of Forest Insects, Bureau of Entomology, United States Department of Agriculture* [2]

INTRODUCTION

Hyposoter disparis Viereck, a parasite of the larva of the gipsy moth (*Porthetria dispar* L.) in Europe, was introduced into the United States by the Bureau of Entomology in the course of the early importations of various parasites of that pest made more than 20 years ago. It has become definitely established in part of the area in the New England States which is infested with *P. dispar*, but has remained of little value as a control factor. However, it seems advisable to define as clearly as possible the apparent status of *H. disparis* as a parasite of the gipsy moth, and to present the information concerning its biology and behavior that has been accumulated at the gipsy moth laboratory at Melrose Highlands, Mass.

SYSTEMATIC POSITION AND REVIEW OF LITERATURE

Hyposoter disparis was first described by Viereck in 1911 (*9*)[3] following its introduction into Massachusetts and after attempts to identify it as a previously described European species had proved unsuccessful. It belongs in the tribe Campoplegini, subfamily Ophioninae, family Ichneumonidae, and order Hymenoptera.

In 1912 Howard and Fiske (*3, p. 191*) published a few notes on the life cycle of this parasite under the name *Limnerium disparis;* and in 1929 Burgess and Crossman (*1, p. 49–52*) summarized very briefly the data available at that time on distribution, life history, and introduction into the United States. Otherwise the species has received only incidental mention as a parasite of the gipsy moth.

DISTRIBUTION IN EUROPE

The original description of *Hyposoter disparis* did not record the type locality more definitely than to indicate that it was European. According to notes at the gipsy moth laboratory, the type specimens appear to have come from Kiev, Russia. In addition, the species has been received from various localities in Austria, Czechoslovakia, Poland, Hungary, Yugoslavia, Bulgaria, and Italy. It appears to be most abundant in south-central Europe.

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[1] Received for publication June 18, 1932; issued March, 1933.

[2] The writers are indebted to C. W. Collins, in charge of the gipsy-moth and brown-tail moth investigations of the Division of Forest Insects, Bureau of Entomology, for advice and criticism in the course of this study; to various members of the staff of the gipsy moth laboratory for the accumulation of data concerning the field collections; and particularly to R. Wooldridge, also of the gipsy moth laboratory, for considerable information regarding the habits of the parasite. They are further indebted to R. A. Cushman, of the taxonomic unit of the Bureau of Entomology, for the identification of some of the hyperparasites recorded from *Hyposoter disparis*.

[3] Reference is made by number (italic) to Literature Cited, p. 346.

IMPORTATION AND COLONIZATION IN THE UNITED STATES

The first cocoons of *Hyposoter disparis*, 20 in number, were received from Russia and Austria in 1907. In succeeding years attempts were made to obtain the species in larger numbers, but it was not until 1911 that cocoons were received in sufficient abundance to provide material for colonization. In that year about 125,000 cocoons of the parasite were collected by agents of the Bureau of Entomology in a gipsy-moth infestation at Gioja Tauro, Italy, and sent to the Melrose Highlands (Mass.) laboratory. Owing to severe hyperparasitism and to considerable mortality during the period of hibernation, from this enormous number of cocoons only about 12,500 adults of *H. disparis* were obtained for colonization the following spring. These adults were liberated in four localities in eastern Massachusetts and at one point in southeastern New Hampshire. Approximately 4,700 were released in North Saugus, Mass.; 3,500 in Andover, Mass.; 2,300 in Boxford, Mass.; and 1,000 each in Wellesley, Mass., and Pelham, N. H.

In 1912 another large shipment, consisting of 171,000 cocoons, was received from Gioja Tauro; but extremely heavy hyperparasitism combined with severe mortality due to the drying out of the cocoons during the winter resulted in an almost total loss of this material, and no liberations could be made in 1913.

Importations of parasites of the gipsy moth ceased with the season of 1912 and were not resumed until 1921. Beginning with 1924 and including 1931, small numbers of *Hyposoter disparis* were received each year incidentally with shipments of other parasites of the gipsy moth from central Europe; but the total for this period was only approximately 5,000 cocoons, of which 3,000 were obtained in 1928 and 1,100 in 1929. Nearly all were from Hungary and Yugoslavia. Since 1912 it has been possible to liberate only three small colonies: One, consisting of 500 adults, in Barnstable, Mass., on Cape Cod, in 1929; another, of 1,862 adults, at Attleboro, in southeastern Massachusetts, in 1930; and a third, of only about 200 adults, in Dighton, Mass., in 1931. The second colony consisted in part of material obtained in reproduction experiments at the Melrose Highlands laboratory.

RECOVERIES FROM FIELD COLONIES AND DISTRIBUTION IN NEW ENGLAND

Collections of gipsy-moth larvae were made at all five of the 1912 colony sites during the same season in which the liberations were made and *Hyposoter disparis* was recovered from each point. Since 1912 the intensity of the gipsy-moth infestation at these five localities has fluctuated greatly, and in some years collections for the recovery of Hyposoter could not be made at all of them. However, the collections that have been obtained have shown that the parasite has persisted at three of the colony sites—namely, Andover, Saugus, and Boxford, Mass.—and possibly at a fourth, Pelham, N. H., and that it has dispersed to some extent, although it has at no time been a parasite of much importance at any of the localities from which collections were secured. It has not been recovered at any of the points at which it was released in 1929, 1930, and 1931.

The present known distribution of *Hyposoter disparis* in New England, as indicated by the recovery records at the gipsy moth laboratory,

appears to be confined to a small area in northeastern Massachusetts and southeastern New Hampshire. (Fig. 1.) The towns in this area from which Hyposoter has been recovered are Andover, Boxford, Saugus, Beverly, and Ipswich, Mass.; and Pelham, Derry, Exeter, and Newton, N. H.

DESCRIPTION OF THE ADULT

The female of *Hyposoter disparis* (fig. 2) may be described as follows:

Average length 6 mm. Head strongly transverse; face granularly punctate, coarsely so medially; clypeus delicately granular; antennae much shorter than

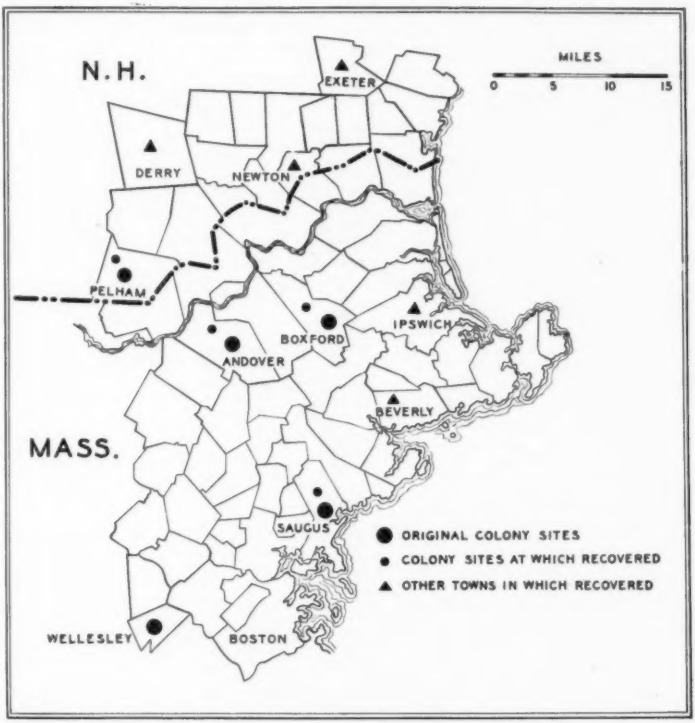

FIGURE 1.—Map showing present known distribution of *Hyposoter disparis*

the body, 32 to 34 segmented; ocell-ocular line longer than diameter of an ocellus. Thorax short and stout, mostly closely granular, the scutellum more weakly so than the mesoscutum; propodeum not extending beyond base of hind coxae, incompletely areolated, areola and petiolar area confluent, the latter a little broadly impressed down the middle and more or less transversely rugulose; anterior wing with a small petiolate areolet. Abdomen somewhat thickened and a little compressed posteriorly, mostly delicately alutaceous; petiole smooth at base; second tergite broadening strongly behind, with the spiracles at about the middle; ovipositor sheaths short, scarcely as long as apical truncature of abdomen. Black; mandibles, except at tips, and the palpi, pale yellow; scape and pedicel of antennae yellowish brown; wings hyaline; legs, including all coxae, testaceous; posterior tibiae slightly dusky at base and broadly black at apex; second abdominal tergite broadly reddish testaceous posteriorly.

In essential characters the male is similar to the female.

Hyposoter disparis is easily confused with an unidentified species of the same genus which is occasionally reared as a parasite of gipsy-moth larvae in Europe. The latter differs, however, in having the antennae 28 to 30 segmented; in the ocell-ocular line being slightly shorter than the diameter of an ocellus; in the less erect areolet of the anterior wing, with the second recurrent joining the cubitus very

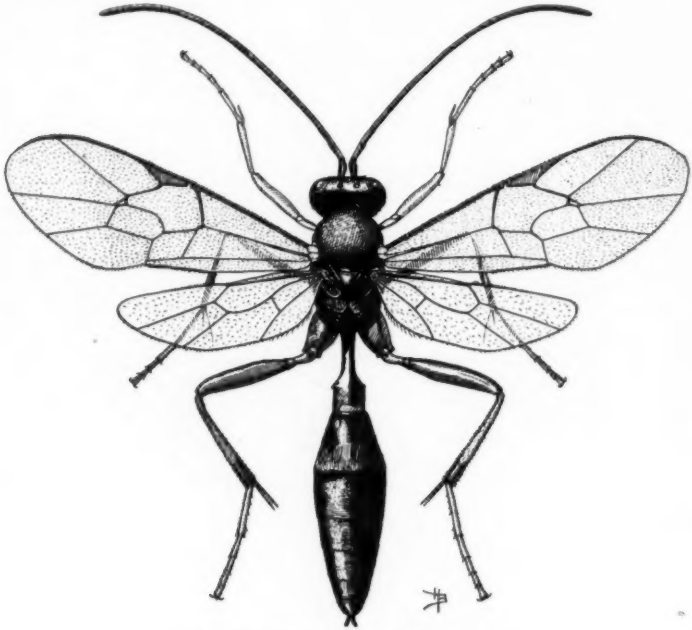

FIGURE 2.—*Hyposoter disparis*, female. × 12

near the second intercubitus; and in having the petiolar area wider and uniformly closely granular and opaque.

LIFE HISTORY AND HABITS

HIBERNATION

Hyposoter disparis hibernates as an adult within its cocoon, the posterior end of the body remaining immersed in the moist meconial discharge. If the meconium dries the parasite dies in the cocoon. Consequently it is important that the cocoons be held sufficiently moist to maintain the semiliquid condition of the meconial discharge. In the field they remain on the surface of the ground throughout the winter, although usually covered to some extent by fallen leaves. At the laboratory, hibernation of *H. disparis* has not been very successful until recently, apparently because the proper moisture conditions have not been supplied. Attempts to simulate field conditions by placing the cocoons on sod or soil and covering them with dried

leaves failed, for the emergence of adults from lots of cocoons held in this manner has been very poor.

In some of the more recent attempts to carry the parasite through the hibernation period, glass cylinders with bottoms of plaster of Paris have been employed. These cylinders are about 3 inches in diameter and range from 3 to 5 inches in height, while the plaster of Paris floors are about half an inch thick. Emergence exceeding 50 per cent was sometimes secured by spreading the cocoons in a thin layer on the floor of such a container and placing the container in a pit partly filled with soil, the cylinder itself being set down about an inch into the soil. The top of each cylinder was covered with cheesecloth and the entire pit sheltered from the rain. However, more or less loss resulted from the molding of the cocoons, and in some cases the mortality was high. Apparently this was due to an excessive amount of moisture drawn up through the plaster of Paris. To obviate this the plaster of Paris was covered with half an inch of sawdust before the cocoons were placed in the container. The results have been highly satisfactory. This method was employed in the hibernation of 2,471 cocoons in 1929, and of these 2,023, or about 82 per cent, produced adults the following spring, which was much the best emergence that had been obtained. Neither the glass cylinder nor the plaster of Paris is essential, for equally good emergence was secured by substituting galvanized-metal cylinders having bottoms of fine wire screening upon which an inch of sawdust had been placed.

EMERGENCE AND MATING

Adults of *Hyposoter disparis* appear to emerge at about the time the eggs of the gipsy moth begin to hatch. In the case of the material hibernated in outdoor cages at the Melrose Highlands laboratory, emergence has usually extended from about April 25 to May 10.

It has been found rather difficult to obtain satisfactory mating in confinement. Most of the earlier attempts were altogether unsuccessful. More recently, however, some degree of success has been attained by employing a cloth-covered cage about 13 inches long, 9 inches wide, and 9 inches high provided with a sliding glass front through which the parasite could be observed and which permitted the easy removal of mating pairs. Best results were secured when not more than 20 to 25 males and 8 to 10 females were used at one time in a cage of this type. Furthermore, it was found that temperatures of 65° F. or higher were required to stimulate the parasites to the necessary activity, and that sunlight was essential. Occasionally females a week old mated in such cages, but the most satisfactory mating was obtained when freshly-emerged females were used with males that were about 3 or 4 days old.

OVIPOSITION

Females of *Hyposoter disparis* oviposit most readily in first and second instar larvae of the gipsy moth. Apparently larvae of the first instar are preferred, although in reproduction experiments at the gipsy moth laboratory larvae of the second instar have usually been employed owing to the greater ease of handling and the somewhat lower mortality in the rearing trays.

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The ovipositing parasites prefer to attack moving caterpillars. Usually, if a female comes upon a larva that is not moving, she slowly encircles it, vibrating her wings and antennae rapidly and occasionally prodding the caterpillar with her ovipositor. Generally the larva soon begins to crawl away, sometimes very rapidly, with the parasite in pursuit. After several additional quick thrusts she finally inserts her ovipositor and deposits an egg, this act requiring about a second. Owing to the habit of the parasites of attacking the host larva in this manner, the eggs are usually deposited in the posterior part of the body. On dissection eggs have frequently been found even in the rectum of the caterpillar, although normally they are placed in the body cavity. Several eggs may be deposited in one host, even in the field, but only one parasite can mature.

POTENTIAL REPRODUCTIVE CAPACITY

An attempt was made to secure some information concerning the number of eggs that may be deposited by a single female of *Hyposoter disparis*. Ten unmated females that had had no opportunity to oviposit were isolated each in a glass vial 8 inches long and 2 inches in diameter, and early each day six first-instar larvae of the gipsy moth were introduced into each vial. Late in the afternoon these larvae were removed and dissected and the eggs deposited by each parasite counted. The total number of eggs deposited by the individual females ranged from 182 to 1,228, with an average of 561 for the 10 parasites. The first death occurred after 12 days and the last after 54 days. In most cases the female continued to oviposit until the day of her death, but the specimen that lived 54 days deposited no eggs after the 34th day, although repeatedly observed to insert the ovipositor in larvae offered after that time. In many cases 50 or more eggs were deposited by a single female on one day, and in one instance as many as 84 were deposited.

FIGURE 3.—Egg of *Hyposoter disparis*. × 120

EGG

The egg of *Hyposoter disparis* (fig. 3) at the time of deposition measures 0.40 to 0.45 mm in length and 0.11 to 0.14 mm in extreme width. It is slightly kidney shaped, smooth, and pearly white. After deposition in the host, the egg gradually increases in size and before hatching attains a length of 0.7 to 0.8 mm and a width of 0.25 to 0.28 mm. Normally the egg stage covers a period of about 7 days, although under unusually low spring temperature this may be extended to 10 days.

LARVA

Only three larval instars seem to have been observed in the case of related Campoplegini that have been studied in some detail. Timberlake (*7*) found only three in *Eulimnerium validum* (Cresson); likewise Tothill (*8*) in his study of *Hyposoter pilosulus* (Provancher) and Thompson and Parker (*6*) in their work on *Eulimnerium crassifemur* (Thomson) mention but three instars. In the course of the present study hundreds of dissections have been made of gipsy-moth larvae which were parasitized by *Hyposoter disparis* at the laboratory, and detailed examinations of the numerous series of Hyposoter larvae

obtained in this way have enabled the writers to distinguish clearly five larval instars in this species.

The first-instar larva is elongate, more or less cylindrical, and smooth, with a strongly sclerotized brown head, and a long caudal appendage, which is a prolongation of the last, or thirteenth, body segment. On hatching, the larva measures about 1.2 mm in length, including the anal appendage, which itself is 0.30 to 0.35 mm long. The mandibles (fig. 4, A) are small, but heavily sclerotized and strongly hooked. Apart from its larger size, the larva of the second instar differs from that of the first principally in its less sharply defined head, differently shaped and less sclerotized mandibles, and the somewhat shorter caudal appendage. The larvae of the third and fourth instars are similar to the second-instar larva, except for the increase in size, and can be distinguished with certainty only by the mandibular differences. In the large quantity of material examined the mandibles in each instar are shown to be remarkably constant in size and form. Identification of the instars was greatly facilitated by the fact that certain larvae, obtained in the course of the numerous dissections, were in the act of molting and accordingly supplied the mandibles of two successive instars.

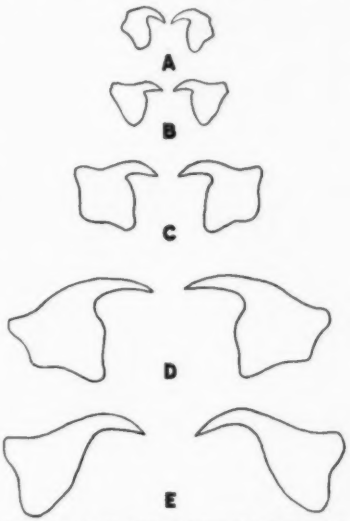

FIGURE 4.—Mandibles of larva of *Hyposoter disparis*: A, of first-instar larva; B, of second-instar larva; C, of third-instar larva; D, of fourth-instar larva; E, of fifth-instar larva. All drawn to same scale

In the mature larva the mandibles are more heavily sclerotized than in the second, third, or fourth instar; the labial ring and the sclerotic framework in the mouth region are brown in color and conspicuous; the body integument is covered with minute tubercles; the antennal, leg, and wing pads can be distinguished; and there are nine pairs of open spiracles, one pair near the posterior margin of the first thoracic segment and a pair on each of the first eight abdominal segments. The caudal appendage is greatly reduced and resembles a short, thick, evenly tapering spine. In length the mature larva measures 8 to 10 mm.

The time spent in each instar varies considerably, being dependent chiefly on the temperature. From the large number of dissections it was determined that 5 to 10 days are spent in the first instar, 2 to 5 days in the second, 2 to 4 days each in the third and fourth instars, and 1 to 2 days in the fifth, while the parasite is still within the host.

The host is killed several hours before the mature parasitic larva emerges and upon the issuance of the latter is left as a flaccid empty skin, practically the entire contents having been consumed. Almost invariably the gipsy-moth larva is in the fourth instar when it is killed by the parasite.

EFFECT OF TEMPERATURE ON DEVELOPMENT

In reproduction experiments conducted in 1929 the period from deposition of the egg to formation of the cocoon ranged from 20 to 33 days, while in 1930 the corresponding figures were 27 to 41. The temperatures during the experiments in 1929 were distinctly higher than during the experiments in 1930. The readings were obtained from the records of a thermograph in the laboratory yard, and daily mean temperatures were computed by averaging the hourly readings. In 1929 the average of the daily mean temperatures for the period concerned was 63.7° F., while in 1930 it was 57.6°. No doubt this difference was, at least in large part, responsible for the difference in the length of the developmental periods for the two years.

FIGURE 5.—Hibernating cocoons of *Hyposoter disparis*. × 2½

COCOON

The cocoon of *Hyposoter disparis* is ovoid in shape and measures about 6 to 7 mm in length and 4 to 4.5 mm in extreme transverse diameter. (Fig. 5.) It is dark gray in color, with a broad, pale gray, transverse band around the middle. An outer layer of comparatively loose silk covers the dense, tough, more or less parchmentlike envelope. Normally the cocoons are formed on the underside of leaves or branches beside the dead host larvae. The attachment to the remains of the host or to the surface of the leaf or branch is so weak, however, that the cocoons invariably, so far as observed, drop to the ground, in most cases apparently within 48 hours after being formed.

PUPA

In order to determine the time of pupation and the length of the pupal stage, numerous dissections were made of cocoons of known ages. Twenty-four hours after completion of the cocoon the short caudal appendage of the mature larva has been retracted and has become shrunken and dark in color. After four days in the cocoon the larva exhibits a slight constriction at the posterior margin of the thoracic region and the developing eyes are weakly discernible. The meconium is usually cast about six days after formation of the cocoon and actual pupation occurs on the ninth or tenth day. The pupa darkens gradually, until at the end of about five days the head and thorax have become black and the base of the abdominal petiole is beginning to darken. Transformation to the adult occurs usually 20 to 21 days after the cocoon is completed, the pupal stage thus covering about 11 days. Normally the adult stage within the cocoon is at-

tained during the first half of July, although emergence does not occur until the following spring, there being but a single generation annually. Very rarely, however, a male adult may emerge the same season in which the cocoon is formed.

HYPERPARASITISM

Because *Hyposoter disparis* has not been abundant in any of the localities in Massachusetts and New Hampshire in which it has been found, and since the cocoons, which drop to the ground soon after they are formed, are difficult to find in the field unless the parasite is present in large numbers, no records have been obtained concerning the extent to which *H. disparis* is attacked by hyperparasites in this country. However, as already mentioned, the cocoons received from Italy in 1911 and 1912 were heavily infested with secondary parasites. The species reared included *Hemiteles areator* Gravenhorst; three unidentified species of the same genus; *Gelis* sp.; *Theroscopus* sp. (?); *Spilocryptus pumilus* Kreichbaumer, var.; *Thysiotorus* sp.; *Theronia atalantae* (Poda); *Itoplectis clavicornis* (Thomson); *I. alternans* (Gravenhorst); *Monodontomerus aereus* Walker; *Monodontomerus* sp.; *Haltichella maculipennis* De Stefani; and *Eurytoma appendigaster* (Swederus). Unfortunately, accurate data concerning the percentage of parasitism by each or all of these species are not available.

ECONOMIC IMPORTANCE

As already mentioned, *Hyposoter disparis* has not yet become an important parasite of the gipsy moth in New England. Year after year, since the original liberations in 1912, the species has been obtained from one or more localities in the course of the intensive rearing work that has been conducted by the gipsy moth laboratory in order to determine the distribution and effectiveness of the introduced parasites of the gipsy moth; but usually it has been reared in such small numbers that the recoveries merely served to indicate that the parasite was still present. However, in 1929, 1930, and 1931 cocoons were obtained in somewhat larger numbers and from a gradually increasing number of localities. In several collections the parasitism by *H. disparis* has been 5 to 7 per cent. Moreover, dissections of a collection of 205 gipsy-moth larvae obtained at Boxford, Mass., in 1930 showed parasitism of 28 per cent by this species.

Members of the staff of the gipsy moth laboratory had observed in certain European infestations of the gipsy moth that cocoons of *Hyposoter disparis* occurred most abundantly in dense woodland, and that they could be found only occasionally along the outer margins or on the edges of clearings. A similar condition in New England infestations within the area where *H. disparis* occurs had been suggested by certain observations. Accordingly, since rearings in 1929 had indicated an increase in the abundance of the parasite at Boxford, Mass., in the spring of 1930 an attempt was made to obtain more exact information on this point. The gipsy-moth infestation at this locality had declined sharply, and consequently only small collections of larvae could be obtained. Nevertheless, the data secured are of interest. In the dense woodland 205 gipsy-moth larvae were collected while at the same time 230 were taken along the edges of this

woodland, all larvae of both lots being obtained from an area comprising not more than 3 acres. All were then dissected. In the larvae collected on the outer margin of the woodland the parasitism by *H. disparis* was only 4 per cent, while those larvae obtained in the shaded parts of the area were 28 per cent parasitized. This is of particular interest since certain other hymenopterous parasites of the gipsy moth have repeatedly been observed to parasitize a higher percentage of the host larvae in the more open growth than in the midst of the woodland.

In the many infested areas in Europe where the gipsy moth laboratory has conducted extensive rearing work during the last 10 years, *H. disparis* has never been observed to be a parasite of more than minor importance. Nevertheless, even species in that category may under certain conditions become the determining factor in the decline of an infestation. Moreover, the fact that *H. disparis* occurred in such enormous numbers in a gipsy-moth infestation at Gioja Tauro, Italy, in 1911 and 1912 that 125,000 and 171,000 cocoons, respectively, could be collected in the two years shows that under some conditions the species may become an important parasite of the gipsy moth.

Little information has been obtained concerning other hosts of *Hyposoter disparis*. At the European station of the gipsy moth laboratory at Budapest, Hungary, large numbers of larvae of many species of Lepidoptera have been reared each year since 1926 in order to obtain information regarding possible alternate hosts of various parasites of the gipsy moth in Europe, but *H. disparis* has never been reared from any of the numerous species collected. In experiments at the Melrose Highlands laboratory the parasite successfully reproduced on larvae of the brown-tail moth (*Nygmia phaeorrhoea* Donovan), but this species does not appear to be so suitable a host as the gipsy moth.

Even the gipsy moth does not seem to be an altogether satisfactory host. Although a single female is capable of depositing a large number of eggs, this potential effectiveness is offset to a considerable extent by failure of eggs to hatch after being deposited in the host or by the early death of the parasitic larva. Large numbers of dissections have shown many parasitized larvae of the gipsy moth to contain dead eggs, and occasionally a dead larva of *H. disparis* surrounded by phagocytes.

PHAGOCYTIC REACTION OF THE HOST

Numerous investigators have observed that internal parasites are sometimes surrounded by phagocytes of the host and most of them have assumed that the parasites had been killed as a result of this phagocytosis, which was considered a defensive measure of the host against the invasion of the parasite. However, Cuenot (*2*) observed that some parasites cause a phagocytic reaction to be set up by the host while others do not; and Pantel (*4, p. 158–160*) concluded that normal healthy parasites do not induce this response, but that the phagocytes accumulate about dead or weakened parasites as they do about inert objects that may find their way into the body of the host. Timberlake (*7*), in his study of the campoplegine *Eulimnerium validum* (Cress.), observed that phagocytosis occurred very commonly when eggs of this parasite were deposited in certain hosts, particularly the

brown-tail moth (*Nygmia phaeorrhoea*) and the eastern tent caterpillar (*Malacosoma americana* Fab.), but not at all when oviposition had occurred in the fall webworm (*Hyphantria cunea* Drury), the normal host of the Eulimnerium. He concluded that normal, or adapted, parasites do not arouse this phagocytic response, which he considered a defensive reaction on the part of the host, while those parasites that are unadapted commonly do. But more recently Thompson (*5*), after wide experience involving observations on the development of many different types of internal parasites, has stated that in his opinion living parasitic larvae occurring free within the body of the host are never attacked by phagocytes, and that the adaptation of the parasite does not consist in the development of substances or structures to give protection against the attacks of phagocytes.

In their study of *Hyposoter disparis* the writers have commonly found eggs of this parasite surrounded by phagocytes of the host. Much more rarely first-instar larvae have been found in this condition. In the course of the experiment, already mentioned, in which an attempt was made to determine the potential reproductive capacity of *Hyposoter disparis*, 3,427 eggs of this parasite were dissected from the gipsy-moth larvae that had been subjected to attack. It is worth noting that of these, 356, or more than 10 per cent, were surrounded by phagocytes within eight hours after oviposition. Futhermore, the dissections of host larvae made with the object of following the development of the Hyposoter larva showed that 13 per cent of the eggs and larvae found were dead and inclosed by phagocytes; and in dissections of several hundred field-collected larvae of *Porthetria dispar*, 42, or about 34 per cent, of the 123 eggs and larvae of *H. disparis* found, were noted as encysted.

The fact that the eggs and larvae of *Hyposoter disparis* so commonly fail to develop in caterpillars of the gipsy moth and become surrounded by phagocytes has suggested that the adaptation of this parasite to *Porthetria dispar* is not complete, especially since no similar condition has been observed with any of the other parasites introduced as agents for the control of this pest. Apparently the egg or larva of the parasite dies before encystment occurs, perhaps because the medium in which the parasite finds itself is not altogether suitable for its development.

SUMMARY

Hyposoter disparis Viereck is a parasite of the larva of the gipsy moth in Europe. It is known to occur in Russia, Poland, Austria, Czechoslovakia, Hungary, Yugoslavia, and Italy, being apparently most abundant in the south-central part of the Continent.

It was first introduced into the United States in 1907 but it was not colonized until 1912. Five colonies comprising 12,500 adults were released in 1912, and the parasite immediately became established. It has dispersed only slightly, however, its present distribution in New England being restricted to a small area in northeastern Massachusetts and southeastern New Hampshire.

The adult parasite is about 6 mm long, with a short, compact thorax, an elongate abdomen, and, in the female, a short, inconspicuous ovipositor. In color it is black with a broad reddish-yellow band on the second abdominal segment, reddish-yellow legs, and hyaline wings.

The species hibernates as an adult in the cocoon, and emergence occurs normally late in April or early in May. Mating under conditions of confinement was best obtained by the use of a small cloth cage held out-of-doors in direct sunlight. Temperatures of 65° F., or higher, were essential. The parasite oviposits most readily in active gipsy-moth larvae of the first and second instars. As many as 1,228 eggs were deposited by one female, and the average for 10 individuals was 561.

The egg is slightly kidney-shaped, smooth, and pearly white; it is about 0.4 mm long when first deposited, but increases in size before hatching. There are five larval instars. The first-instar larva is elongate, cylindrical, with a strongly sclerotized brown head and a long caudal appendage. When mature, the larva measures 8 to 10 mm in length, the mandibles and the framework of the mouth region are heavily sclerotized, there are nine pairs of open spiracles, and the caudal appendage has become greatly reduced. The period from egg deposition to formation of the cocoon ranges from about 20 to about 40 days, and depends in large part on the temperature. The cocoon is ovoid, about 6 to 7 mm long, dark gray in color, with a broad, complete, transverse pale-gray band at the middle. Pupation occurs 9 or 10 days after cocoon formation, and transformation to the adult 10 or 11 days later. There is but one generation annually.

Hyposoter disparis has, on rare occasions, been observed to be a very abundant parasite of the gipsy moth in Europe, but it has not yet become an important control agent of that pest in New England. Apparently the parasitism is heavier in dense woodland than in open growth or on the outer edges of wooded areas.

The potential effectiveness of the species as a parasite of the gipsy moth is offset to a considerable extent by failure of eggs to hatch or by early death of the parasitic larvae. From 10 to more than 30 per cent of the eggs and young larvae of the parasite dissected from host caterpillars that had been attacked in the laboratory and in the field were found to be dead and surrounded by phagocytes of the host. This suggests incomplete adaptation of the parasite to this host.

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CURVED-LINE RELATIONSHIPS BETWEEN CERTAIN SHORT-PERIOD EGG YIELDS AND ANNUAL EGG PRODUCTION IN SINGLE-COMB WHITE LEGHORN FOWLS[1]

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INTRODUCTION

Scientific investigators and practical poultry producers have long since agreed that capacity to produce increased numbers of eggs is, at least to some considerable extent, inherited. It is obvious that the successful poultry farmer must (1) secure as high an average egg yield per year per bird as is possible without impairing the health of the birds, and (2) employ certain principles of selection and breeding to accomplish this. Poultrymen recognize differences between the individual birds in their flocks in egg-producing capacity. They realize that criteria of selection must be used in establishing breeding flocks from which future layers are to be produced. The trap nest has been employed as a mechanical means of securing a measure of egg production. The exact record of daily egg yield for each individual for the entire pullet year of production has proved to be a useful and reliable criterion in selecting breeding stock. The trap-nest method, however, is so laborious and expensive that its general use is prohibitive, therefore materially reducing the number of accurately recorded birds from which to choose breeders.

The purpose of the present study, which is based on correlations previously reported between winter-season egg yield and yearly total egg production, was to investigate the nature and type of such correlation, to make a similar correlation with another short period, and to determine the possibilities of drawing up a workable, practical breeder-selection program.

REVIEW OF LITERATURE

A survey of the literature indicates that several workers have noted that some significance attaches to the egg-yield record made during the winter season; that is, during the first three or four months of the first, or pullet, laying year.

Ball and Alder (*1, p. 53–54*)[2] in 1917 made some interesting observations:

> Environmental factors influence the records of the pullet year more than that of later years and influence flocks making low records more than those making high ones. * * * Winter egg production of flocks is more variable than annual production. This variation seems to be closely correlated with environmental factors. * * * The correlation between "winter" production and annual production was subject to wide and irregular fluctuations in the different flocks, without reference to the height of production. * * * The average correlation was 58 per cent. The ("winter") period as used does not seem accurately to represent a biological entity, but is made up of the end of one period and the beginning of another.

[1] Received for publication Apr. 26, 1932; issued March, 1933. Journal Series Paper of the New Jersey Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 358.

This contribution from the Utah station was part of a rather extensive series of studies on the economic significance of various phases of egg production over a period of years. This work did much to impress upon poultry breeders the differences between individual fowls.

Harris, Blakeslee, and Kirkpatrick (*2*) in 1918 reported that in the Single-Comb White Leghorn fowl, under reasonably uniform conditions of breeding and management (they used Storrs egg-laying-contest records), there was a significant positive correlation between the number of eggs laid by a bird in any month of the year and the number of eggs laid during the whole year. They further observed that there was a more intimate correlation between the egg production of the autumn and winter months at the beginning and end of the contest year than between the egg production of these months and that of the spring and summer months.

Harris, Kirkpatrick, Blakeslee, Warner, and Card (*3*) in 1921 again presented evidence to the effect that annual egg production for the pullet year might "with a high degree of accuracy" be predicted when the known yield for one, two, or three months was available. They suggested that full-year trap nesting, admittedly expensive in operation, might not always be necessary.

Harris and Lewis (*4*) in 1922, using New Jersey egg-laying-contest data, similarly indicated a sufficient degree of association between certain short-period trap-nest records and annual egg-production records to warrant their use for prediction purposes.

Hervey (*7*, *8*) in 1923 and 1924 continued studies of the correlation between short-period egg yield and annual total yield and described such relationships in a way that would be useful to practical poultry breeders. Hervey (*7*) reported a coefficient of correlation of $+0.7752 \pm 0.0132$ between winter egg yield and annual total, on the basis of which, using a straight-line equation, he offered a regression equation for prediction of annual egg production when the winter record was known, as follows: Y (annual) $= 1.411X + 116.8$.

Hervey (*7*, *p. 6*) stated:

> Precedent for the use of the straight line has been set by Harris in a series of reports with other authors, * * * not with the idea that regression is always linear when production is considered, but in the absence of a large amount of data to prove the contrary its use seems justifiable.

Hays, Sanborn, and James (*6*) in 1924 reported on breeding work in the Rhode Island Red in which they indicated a coefficient of correlation of $+0.6214 \pm 0.0142$ between winter yield and annual total and stated that the winter record was of significant value in the selection of pullet breeders. Hays (*5*) later presented evidence of a highly significant degree of association between winter and annual egg production.

These, and other workers, have paved the way for drawing up a practical breeder-selection program based on the use of short-period trap-nest records. So far as the present author has been able to determine, previous workers have all used a straight line to express relationship between the short-period trap-nest record (in all cases up to this time this short period has been either a month, or the winter season, except in the work of Hervey (*7*)). Practical poultrymen have at times expressed dissatisfaction with the use of such straight-line equations, feeling that the results were not sufficiently accurate to be

used in substitution for full-year records. Some of these points of view raised questions which a review of the previous work did not seem fully to answer. Several poultrymen have pointed out that such studies as these would possibly have increased interest and value if the information thus obtained could be applied on poultry-breeding plants where whole flocks were placed under trap nest on set dates and where it was not practicable to get individual records from the first egg. Hays et al. (*6*) of course had reported on a certain strain of the Rhode Island Red. They also used data from the first egg.

MATERIALS AND METHODS

During the two years following October 1, 1929, several thousand pullets were accurately trap nested and their respective egg yields definitely recorded at one or another of the three official egg-laying contests operated under the management of the Department of Poultry Husbandry, New Jersey Agricultural Experiment Station, and located at Paterson, Flemington, and Vineland, N. J. Included in this number were several thousand Single-Comb White Leghorns, a variety used extensively in the commercial table-egg industry. Upon examination of the available trap-nest records it was found that there were complete full-year (pullet-year) records at hand for 3,937 individuals, each one of which had lived throughout the first contest year (October 1 forward for 51 weeks). These individuals, which have been used in this study, were found distributed through 375 flocks. The flocks in the main represented as many different lines of breeding, since each flock in the egg-laying contest consisted of 16 birds sent by the poultry breeder. The sample is considered representative of the Single-Comb White Leghorn variety in the Eastern States. The various probable errors computed during the work tend to give evidence that this statement is correct.

It should be recalled that the pullets sent to egg-laying contests are in the great majority of cases so selected that they are in a stage of maturity which will bring them into laying condition at a time very near to the opening of the contest year. Thus it is safe to assume that the records here used were gathered from birds so hatched and reared as to bring them into production on approximately October 1. It is this definite group of birds on which this study has been made, and, therefore, it is for such type of birds that the results would naturally apply.

The egg yield during the first 120 days of the egg-laying-contest year—that is, from October 1 through January 28—was considered as the winter period, and the egg yield during this period (although the limits of the period were arbitrarily set) was used as a measurement of the pullet's capacity to produce eggs during the early weeks of the first production year. It was assumed that (1) pullets reach the date of first egg, or sexual maturity, under average conditions, physically strong and vigorous; (2) pullets are likely to produce eggs chiefly as a result of inherited capacity during the earlier weeks of the production year; and (3) production will probably be less affected by environmental factors during this winter period than during the full-year period. If these premises are correct, the record of egg yield during the winter period takes on an unusual significance and gives promise of being an economically useful criterion of selection. Evidence already submitted substantiates this view.

The egg yield during the last 30 days of the contest year—that is, during the period between August 25 and September 23, inclusive—was adopted as a short period at the end of the pullet year of production which might give a practical picture of a tendency to maintain egg production well through the summer-fall season. In the industry it is generally recognized that pullets which are able to keep up egg yield well through the hot summer months and into September are usually among the more prolific egg producers of the flock. Because of market-egg prices during this summer-fall period and because of the general labor situation on the average farm at that time egg yield during August and September is of considerable importance. The dates are arbitrarily selected.

Prof. C. S. Platt of the New Jersey station suggested to the author that the addition of the two short-period records just referred to might become a still more useful criterion of selection than either used separately, and that the essential data obtained might not be impracticable for general use. For purposes of brevity this combined period is called the "120+30" record.

The following series of correlations are apparently essential:

(1) Between winter egg yield (X_1) and annual total egg production (Y)
(2) Between winter egg yield (X_1) and the last 30-day yield (X_2)
(3) Between last 30-day yield (X_2) and annual total egg production (Y)
(4) Between annual egg production (Y) and winter egg yield (X_1), with the last 30-day egg yield (X_2) held constant.
(5) Between the "120+30" yield (X) and annual total egg production (Y).

After the nature of these several correlations was determined, a series of regression equations for prediction purposes was derived. These are given later in this paper.

It was planned to formulate, if possible, a practicable selection program for the use of poultry breeders, embodying the use of short-period trap-nest records and their relationship to the full-year trap-nest record.

ANALYSIS OF EGG-PRODUCTION RECORDS

Methods of statistical technique were applied to the available 3,937 pullet-year egg-production records in an effort to obtain the necessary facts and figures for the interpretation of these records. The results are presented as problems 1, 2, and 3, respectively. In all problems the value of $N=3{,}937$ cases.

Problem 1. Correlation between winter egg yield and total annual egg production, X_1 and Y, respectively:

		For X_1	For Y
(1)	Mean, with its probable error	[3] 56.6	± 0.2474	202.96 ± 0.8241
(2)	Standard deviation, with its probable error	21.1	± 0.1607	51.71 ± 0.3928
(3)	r_{xy}	$+0.6352 \pm 0.0064$		
(4)	η_{yx}	$+0.6641 \pm 0.0033$		

(5) Blakeman's test for linearity (short) showed $12.1538 > 4.047$, and therefore a curvilinear relationship was indicated between X_1 and Y.
(6) Regression equation (straight line, i. e., $Y=a+bX_1$) was found to be, with its standard error of estimate, as follows: $Y=1.553\,X+115.032 \pm 30.8435$.
(7) Curvilinear regression equation (log $Y=a+b$ log X_1) was found to be log $Y=1.24851+0.6143$ log X_1.
(8) Class interval for $X_1=10$ eggs, and for $Y=25$ eggs.

Problem 2. Correlation between winter egg yield and total annual egg production, X_1 and Y, respectively, with last 30-day egg yield (X_2) held constant:

	For X_1
(1) Mean, with its probable error	[3] 19.4 ± 0.0656
(2) Standard deviation, with its probable error	6.1 ± 0.1473
(3) $r_{12.3}$	$+0.7487$, when 1 refers to annual egg production, 2 to winter yield, and 3 to last 30-day yield.
(4) r_{12}	$+0.6352 \pm 0.0064$
r_{13}	$+0.6024 \pm 0.0069$
r_{23}	$+0.0610 \pm 0.0681$

(5) Partial regression equation ($Y = b_{12.3}\ X_1 + b_{13.2}\ X_2 + C_1$) was found to be $Y = 1.4692\ X_1 + 4.7905\ X_2 + 26.9437 \pm 18.4063$.

Problem 3. Correlation between the "120+30" egg yield and total annual egg production, X and Y, respectively:

	For X
(1) Mean, with its probable error	61.0584 ± 0.4584
(2) Standard deviation, with its probable error	42.6404 ± 0.3241
(3) r_{xy}	$+0.6711 \pm 0.0088$
(4) η_{yx}	$+0.7150 \pm 0.0078$

(5) Blakeman's test for linearity (short) showed $8.84084 > 4.047$, and therefore a curvilinear relationship was indicated between X and Y.

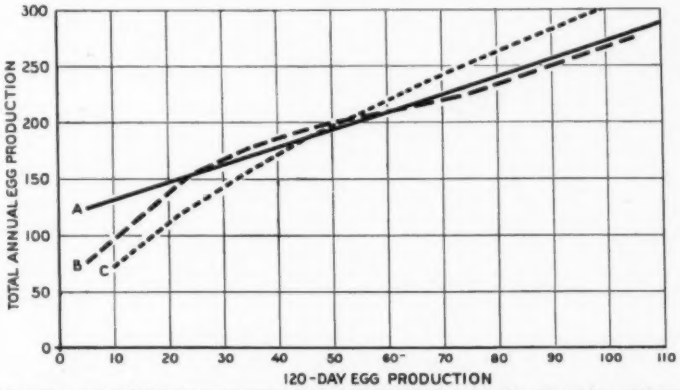

FIGURE 1.—Correlation between egg yield during first 120 days (as X) and total annual egg production (as Y): A, Regression of Y on X (straight line, $Y = a + bX$); B, line connecting means of columns; C, regression of Y on X (curved line, log $Y = a + b$ log X)

(6) Regression equation (straight line, i. e., $Y = a + bX$) was found to be, with its standard error of estimate, as follows: $Y = 0.8138\ X + 153.2667 \pm 28.4184$.

(7) Curvilinear regression equation (log $Y = a + b$ log X) was found to be log $Y = 1.2324 + 0.64208$ log X.

(8) Class interval for $X = 10$ eggs.

The results here indicated are of considerable interest, especially in that (1) the size of N (3,937 cases) is very much greater than has been the case in earlier work and is sufficiently great to merit attention as a fair cross section of the possible universe, (2) a curvilinear relationship is indicated as existing between winter egg yield and the annual egg-production total, whereas in earlier reports only linear relationship has been dealt with, (3) a distinctly curvilinear relationship is shown between the "120+30" egg yield and the annual egg-production total, and (4) that an even greater and more significant correlation exists between the "120+30" egg yield and the annual yield than between winter yield and annual production.

[3] All values were carried to the fourth decimal place for use in computing.

Figures 1 and 2 show certain of these data.

The poultry population of an egg-laying contest is closely akin in type to that which would be found on the poultry-breeding farms from which the contest-flock birds have come. The poultry breeder selects certain of his maturing pullets to be trap nested, part of them are sent to a contest and part are trap nested at home. The 3,937 individuals used in this investigation are considered, therefore, as representing a fair cross section of Single-Comb White Leghorn pullet layer stock. Figure 3 shows the distribution data of the 3,937 individuals as to egg yields during the several short-period intervals and during the full-year period.

In each instance the points located represent the frequencies at the respective midpoints of the chosen intervals. Frequencies have been plotted on the ordinate, and egg yields along the abscissa.

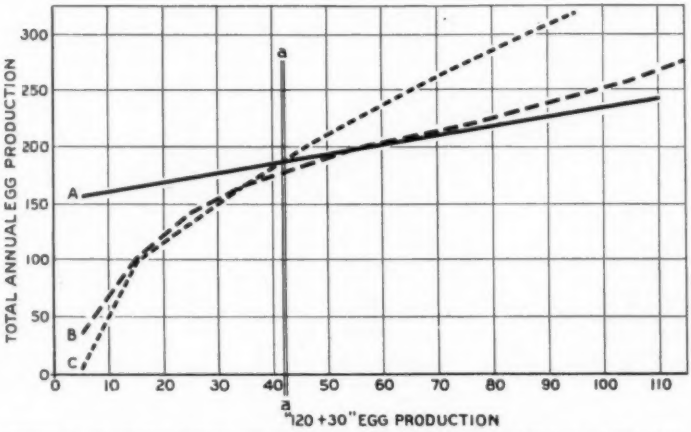

FIGURE 2.—Correlation between egg yield during first 120 days plus last 30 days (as X) and the total annual egg production (as Y): A, Regression of Y on X (straight line, $Y=a+bX$); B, line connecting means of columns; C, regression of Y on X (curved line, $\log Y=a+b \log X$); *aa*, division line (see text)

DISCUSSION OF EGG PRODUCTION RECORDS

Poultry breeders are apparently using egg-laying contests in a serious attempt to identify future breeders of increased egg-production capacity. This is indicated in the distribution of cases as to annual egg production. A study of Figure 3 shows that in these 3,937 cases 84 per cent of the individuals produced between 150 and 274 eggs, inclusive, 13 per cent produced between 0 and 149 eggs, inclusive, and only 3 per cent produced more than 274 eggs during the contest year. These figures indicate a very satisfactory proportion of highly economical and profitable egg producers. The class between 200 and 224, inclusive, was the modal class and contained 949 individuals. The study dealt, therefore, with types of fowls which, other things being equal, might furnish at least a fair percentage of eligible breeders.

In the winter egg-yield distribution the modal class (10-egg interval) was that between 50 and 59, inclusive, with 652 cases, but the class next below the modal class and the two classes just above it were

close to it in frequencies, the four classes showing 558, 652, 639, and 577 cases, respectively. This span between 40 and 80 eggs as winter yield included 61.62 per cent of the total number of pullets in the flocks. This unusually high winter-production tendency was considered as indicating a generally high quality of birds in this study.

Further evidence of the high quality of these 3,937 birds was found in the third distribution curve, which indicated that as to "120+30" egg yield the modal class (again a 10-egg interval used) was that between 60 and 69, inclusive, with 604 cases, 66.32 per cent of the birds falling within a range between 50 and 100 eggs for this "120+30" period.

RELATION BETWEEN WINTER EGG YIELD AND ANNUAL TOTAL EGG PRODUCTION

The plotting of the means of each class interval in winter-yield distribution, as to annual egg production (fig. 1, B) at once intimated that a somewhat curvilinear relationship apparently existed between these two categories. The Blakeman test for linearity further con-

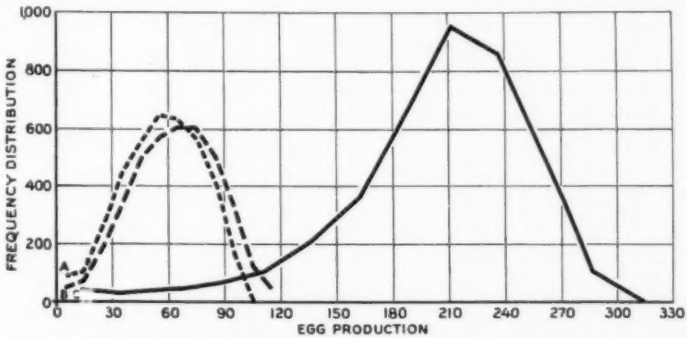

FIGURE 3.—Distribution of individuals (3,937 cases) as to egg production: A, winter egg production; B, first 120 plus last 30-day egg production; C, annual egg production

firmed this belief. Finally, the plotting of the regression lines (fig. 1, A and C, respectively) and a comparison of their locations with respect to the line connecting the means of the columns seemed to warrant the conclusion that in these 3,937 Single-Comb White Leghorn pullets the correlation between winter egg yield, as represented by the first 120-day egg yield, and the total egg yield for the first 51 weeks, or contest year, was distinctly curvilinear.

The correlation ratio, the η_{yx} value of $+0.6642 \pm 0.0033$, has been deemed a more accurate expression of the degree of relationship between these two categories than is the r_{xy}, or coefficient of correlation, of $+0.6352 \pm 0.0064$.

No very material advantage is to be gained through the use of the logarithmic-regression equation, $\log Y = 1.24851 + 0.6143 \log X$, over that of the straight-line equation, $Y = 1.553\ X + 115.032$, in the group having a winter egg yield approximately between 40 and 79 eggs, inclusive, since both equations would predict within a reasonable degree of the same thing. In those cases in which the winter yield is below 40 and above 80 eggs, the logarithmic prediction formula is more reliable than the straight-line equation.

It is not suggested that the formula presented necessarily gives the closest approach to the curvilinear data of this problem. Future studies of other possible formulas may determine a regression equation which will adequately fit the data presented. At the moment, with particular emphasis upon practical usage, the use of the 2-regression equation as indicated in the text and in Figure 2 is offered as a workable proposition at least pending the finding of some single-regression equation which in itself would more nearly fit the data.

The author feels so strongly that the 3,937 cases used in this investigation represent a fair cross section that he would be inclined to use the graphic method of predicting annual egg production from known winter yield rather than either of the mathematical formulas here presented. He suggests that the curve shown in Figure 1, B might be thus used advantageously. The practical poultry breeder, on January 28 would stop trap nesting and recording and add up the individual records for the winter months. Then, having for each individual bird a known winter-season egg-yield record he would find that known record along the x, or base line. The intersection of the line from that point with curve B, in Figure 1, indicates the predicted or expected annual egg production. For example, if the known winter yield is 60 eggs, one reading direct from the graph would predict an annual production of 210 eggs, if the winter yield is 40 eggs, the annual production would be about 180 eggs, and if the winter yield is 75 eggs, the annual production would be about 230 eggs, etc.

If the winter egg yield may be taken as a reasonably fair indication of the inherited capacity of fowls to produce eggs, the economic significance of this winter egg-yield record is at once apparent. It permits the poultry breeder to cease the expensive and laborious task of trap nesting and recording after January 28. It furnishes him with sufficiently accurate and complete information concerning each individual to allow him to group or classify his fowls with a high degree of skill. Upon this premise it is suggested that the average flock thus trap nested during only the 120-day period after October 1 might be divided into three groups, for breeding purposes, as follows:

Group 1 would include pullets producing from 50 to 69 eggs, inclusive, during their first 120 days, a class with a range of predicted annual egg-production values from approximately 196 through 240 eggs (using logarithmic equation). All birds that do not come up to this minimum of 50 eggs during the winter period should be discarded.

Group 2 would include those pullets producing from 70 to 89 eggs, inclusive, a class with predicted annual egg-production totals between approximately 240 and 280 eggs.

Group 3, the smallest group, in numbers, would include those pullets producing more than 89 eggs during the winter period, or having a predicted annual record of 280 or more eggs. This group would represent the highest degree of laying efficiency found in the trap-nested stock.

Of course, such selection and grouping as is here suggested would be made only if other things were equal, such as health, type, and size, and, perhaps, standard quality.

If the trap nesting must stop with the winter season, this plan is workable.

RELATION BETWEEN "120+30" EGG YIELD AND ANNUAL PRODUCTION

Hervey (7) found a significant correlation between the summer-fall period egg yield and the total egg production for the year, expressed by the coefficient $+0.7860 \pm 0.0135$. Other investigators have indicated that prolonged production throughout the pullet year period was an indication of high egg-yield capacity. As explained earlier in this paper, the arbitrary 30-day period between August 25 and September 23 has been chosen as a measure of prolonged egg yield. It was suggested that this measurement added to that of the winter season might furnish a still better criterion of selection than did winter yield alone, or summer-fall record alone.

From a practical point of view, this seems sound. The poultry breeder can economically and easily trap nest a fairly large number of pullets during the first four months, or through January 28. He must then stop that work because of the heavy demands of the chick hatching and rearing season. He must cut down on expenses as well.

As summer wanes, however, the young chickens need little care. The poultryman's time is less filled and he finds that he can again take up some trap nesting. He will start trap nesting the new pullets as October approaches. He can easily start to operate the trap nests on the laying flocks during late August and economically and efficiently trap nest some of the pullets which he trap nested during the preceding winter season.

The second part of this investigation consisted in an attempt to find out whether or not it would be worth while to trap nest during this late 30-day period. By similar tests to those applied to the winter-season data, the relationship between the "120+30" day egg yield and annual egg production was found to be distinctly curvilinear, as shown by Figure 2.

A study of the three curves in Figure 2 reveals a considerably more interesting and probably a much more serviceable measurement of egg-production capacity than was found when only the winter egg yield was used. In the first place, the degree of association between the "120+30" day egg-yield record and that of the full year is greater than that in the other case. In this instance the value of r was $+0.6711 \pm 0.0088$, and that of η_{yx} was $+0.7150 \pm 0.0078$. This gives an increased value to the predictions based upon that relationship. The curvilinear nature of the relationship is sharply defined, and the line connecting the means of columns tends to coincide with the two regression lines.

After a careful study of Figure 2 and its formulas, used on innumerable cases for prediction purposes, the following suggestions are offered: (1) When the egg yield during the "120+30" period is below approximately 42 eggs, the relationship which that yield bears to the annual total egg production is very distinctly curvilinear in character. The predicted annual production attained by use of the logarithmic formula, $\log Y = 1.2324 + 0.64208 \log X$, and that obtained by direct reading through use of curve B, in Figure 2, are very closely similar. The differences are, at least from the standpoint of the practical poultry breeder, negligible. (2) A division line can be established, therefore (*aa* in fig. 2), below which point the logarithmic equation is to be used, if for any reason it is desired to predict annual production on birds of that degree of egg-laying capacity. (3)

Above the line *aa* the straight line equation, $Y=0.8138X+153.2667$, should be used for prediction purposes, or a direct reading from curve B, either of which would render approximately the same results.

Although for the practical poultryman interested primarily in finding a method of using a short-period trap-nest record as a criterion of selection, the linear equation just referred to would be sufficient, it is of interest to poultry science to have the situation as it stands below the *aa* line defined.

The foregoing discussion has been limited to the possible statistical and economical usefulness of (1) the winter egg-yield record alone, and (2) the combination of the winter record with that of the last 30 days of the laying year, i. e., the "120+30" period record. It is possible that, if, instead of combining the winter and the last 30-day records into one measurement, each is used separately, still closer prediction of annual egg production might be made. Therefore, the study made as problem 2 was undertaken. A partial correlation was worked out, and a partial regression equation evolved. The separate use of the two short-period trap-nest records offers a more accurate and exact means of prediction than does either of the other two formulas and is no more difficult to use. Scientifically, it is, perhaps, an interesting and worth-while example of the usefulness of the partial correlation and regression principle to poultry-science problems.

SUMMARY

In the case of 3,937 Single-Comb White Leghorn pullet layers studied as to first-year egg-production capacities, a highly significant positive correlation of curvilinear type was found to exist between the egg yield during the first 120 days after October 1 (approximately date of first egg) and total egg production for the contest year (51 weeks after October 1).

A still more significant positive curvilinear correlation existed between the "120+30" day egg yield and annual total egg production.

If both winter egg yield and yield during the 30-day summer period are available, the practical poultry breeder has an efficient means of predicting annual total egg-producing capacity in the partial regression, or prediction equation here given.

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INHERITANCE IN ALBIT WHEAT OF RESISTANCE TO BUNT, TILLETIA TRITICI[1]

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INTRODUCTION

Albit wheat (Washington No. 2517, C. I. No. 8275, and registration No. 258) is the result of a cross between Hybrid 128 (female) and White Odessa (male), made in 1920 by E. F. Gaines. It is a club wheat with awnless spikes, glabrous white glumes, and soft white kernels.[2] In morphological characters it is like Hybrid 128[3] (fig. 1), but in its resistance to bunt (*Tilletia tritici* (*Bjerk.*) Wint. and *T. levis* Kühn) it resembles White Odessa.

In 1931 the senior author[4] reported that Albit showed the same reaction to bunt as White Odessa, its bunt-resistant parent. "It appears," he stated, "that Gaines has completely transferred the resistance of White Odessa to a segregate having morphological characteristics similar to those of the susceptible parent, Hybrid 128."

Martin, White Odessa, Banner Berkeley, Albit, and Regal wheats have been found by the senior author[5] to be similar in their resistance to *Tilletia tritici* and *T. levis*. Briggs[6] has shown that Martin, White Odessa, and Banner Berkeley have one main dominant factor for resistance to *T. tritici*.

EXPERIMENTAL DATA

Data previously reported by the senior author[7] showing the reaction of White Odessa and Albit wheats to 43 different collections of bunt are presented graphically in Figure 2. This figure shows that the two varieties are similar in their reaction to all 10 of the forms of bunt (included in collections) used in earlier tests. Both were resistant to 29 collections and susceptible to 14. The one discrepancy, apparent susceptibility of White Odessa to collection 7c and resistance of Albit, was cleared up in 1929 when it was found that both varieties are resistant to this collection. Other trials in 1930 and 1931 also indicate that these two varieties are identical in their ability to resist bunt.

In 1928 several crosses were made between Hybrid 128 and Albit. The F_1 plants were grown in the greenhouse in 1928–29. The F_2 plants were grown in 1929–30 from copper-carbonate treated seed so that the F_3 generation should contain both susceptible and resistant

[1] Received for publication Apr. 13, 1932; issued March, 1933. Contribution from the Department of Farm Crops, Oregon Agricultural Experiment Station. Paper No. 183 of the Journal Series.

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[7] BRESSMAN, E. N. Op. cit.

FIGURE 1.—Typical heads of White Odessa (A) and Hybrid 128 B). A cross between these two, made by E. F. Gaines in 1920, yielded a segregate, Albit (C), which carries the morphological characters of the female parent (Hybrid 128) and the bunt resistance of the male parent (White Odessa)

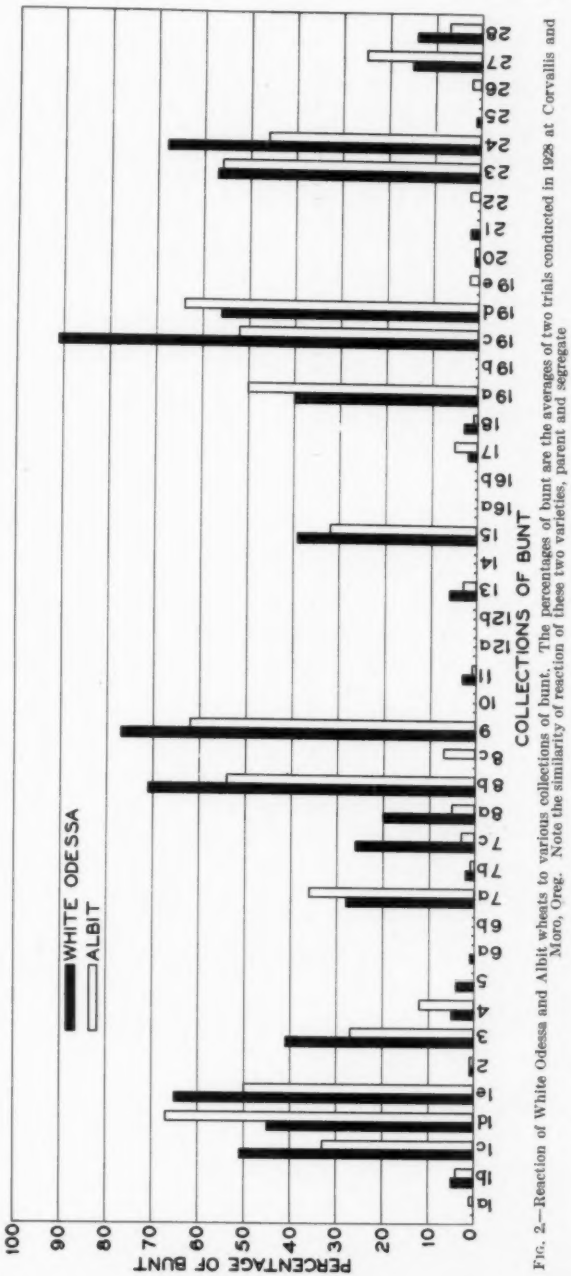

FIG. 2.—Reaction of White Odessa and Albit wheats to various collections of bunt. The percentages of bunt are the averages of two trials conducted in 1928 at Corvallis and Moro, Oreg. Note the similarity of reaction of these two varieties, parent and segregate

segregates. In the fall of 1930 the seed for the F_3 generation was heavily coated with inoculum from bunt collection No. 12a. This collection was originally obtained from Tucson, Ariz. It is a form of *Tilletia tritici*, classified as physiologic form VIII. This collection was used as inoculum because the Hybrid 128 parent is susceptible and the Albit parent is resistant.

No bunt appeared at Corvallis on Albit when it was inoculated with collection 12a (Table 1); but in 1929 at Moro 2 bunted heads out of a total of 505 were found in this variety. No significance is attached to this fact, however, for a few bunted heads are commonly found in a resistant variety. Hybrid 128 in all tests was susceptible to collection 12a. At Moro in 1929 this collection produced 63 per cent infected heads in Hybrid 128.

White Odessa was inoculated with collection 12a in 1929 only. In these trials 200 heads of White Odessa were counted at Corvallis and 300 at Moro, but no bunt was found.

TABLE 1.—*Percentage of bunt in the parent varieties when inoculated with bunt collection 12a, Corvallis, Oreg.*

Variety	Percentage of bunt in—				
	1928	1929	1930	1931	Average
Albit	0	0	0	0	0
Hybrid 128	43	40	91	82	64

In the Hybrid 128 × Albit crosses, the F_1, F_2, and F_3 progenies were all identical in morphological characters with the parent varieties. In other words, there was no segregation for these characters. In fact, the only method of determining that hybridization had been effected was through the segregation for bunt resistance. This segregation is shown in Table 2. Each row, fall sown in the field at Corvallis, was 16 feet long and contained about 60 plants. The F_3 generation was represented by four families and a total of nearly 5,000 plants. One family did not segregate for bunt resistance, indicating that it was not a hybrid. The results of its smutting, therefore, are not included.

TABLE 2.—*Distribution of the F_3 rows of the cross, Hybrid 128 × Albit, into 10 per cent classes for bunt infection, Tritici species, collection 12a, physiologic form VIII, Corvallis, Oreg., 1931*

Hybrid family No.	Number of rows having indicated percentage of plants infected										Total rows
	0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100	
1	5	2	5	1	2		1	4	4		24
2	3		1			1	2	1			8
3	4	3	1	4					2	2	16
4	4	4	7	5	1			2	3	2	28
Total	16	9	14	10	3	1	3	7	9	4	76

P=0.72.

In the crosses between Hybrid 128 and Albit the distribution of F_3 rows into 10 per cent classes for bunt infection agrees closely with a 1:2:1 ratio when the 0–10 class is considered as resistant and the classes above 70 as susceptible. In the resistant class there are 16 rows, in the susceptible class 20, and in the segregating class 40. On the basis of a 1-factor difference, there should have been 19, 38, and 19 rows, respectively, in the three classes. Since $P=0.72$, a very satisfactory agreement with the 1:2:1 ratio is indicated. The value of P was obtained from Fisher.[8]

One family, No. 3, shows a perfect segregation of 1:2:1 with 4 resistant rows, 8 segregating, and 4 susceptible in a total of 16 rows. In fact, all four families show a satisfactory agreement with a 1:2:1 ratio. The F_3 rows are plant rows.

Briggs [9] states that there may be more than one factor for resistance in White Odessa wheat. The results herein reported indicate that White Odessa has only one factor for resistance and that it applies only to certain physiologic forms of bunt, namely, Nos. II, III, V, VI, and VIII of the senior writer's classification.[10] This factor (*MM*) has been transferred from White Odessa to Albit, so that both varieties contain the identical factor. These varieties, however, contain no factor for resistance to forms I, IV, VII, IX, and X. Hybrid 128 is entirely susceptible and carries no factor for resistance to any of the physiologic forms.

SUMMARY AND CONCLUSIONS

Albit wheat contains one main dominant factor for resistance to certain physiologic forms of bunt. This factor, *MM*, is the same as that carried by its male parent, White Odessa.

Apparently the factor *MM* is fairly well distributed, for it has been found by Briggs (who designated it) or by the writers in Martin, White Odessa, Hussar, Banner Berkeley, Albit, and possibly Regal wheats. These wheats differ in species, size and shape of head, color of kernel, color of chaff, bearded condition, color of straw, and other characters.

Albit wheat contains morphological characters similar to its female parent, Hybrid 128. Crosses between Albit and Hybrid 128 do not segregate for morphological characters, but they do segregate for bunt resistance and possibly for other factors such as yielding ability. Apparently Hybrid 128 carries no factor for bunt resistance.

[8] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 2, rev. and enl., 269 p., illus. Edinburgh and London. 1925.
[9] BRIGGS, F. N. Op. cit. (Footnote 6, first reference.)
[10] BRESSMAN, E. N. Op. cit.

THE VITAMIN C CONTENT OF THE WINESAP APPLE AS INFLUENCED BY FERTILIZERS [1]

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INTRODUCTION

The value of many fruits as a source of vitamin C is widely accepted. Much information is available concerning the occurrence and stability of vitamin C in food materials. Information is incomplete, however, concerning the possible influence of various factors in the field, such as cultural operations employed in growing the product. The application of mineral fertilizers may measurably affect tree vigor, the relation of leaf area to fruit borne, the size, color, and time of maturity of individual specimens, and even their chemical composition, especially the mineral content.

Bracewell, Hoyle, and Zilva (*2*)[3] found that varieties were significantly different in vitamin C potency, but they reported no indication that the age of trees, soils, or season had any bearing on the antiscorbutic potency of the apple. A publication from this same laboratory in 1931 (*4*), upon vitamin C and the nitrogen content of apples, reported no conclusive evidence of a relationship between nitrogen content and vitamin C value.

It was believed that a study of the possible influence of the application of a complete fertilizer (N, P, and K) upon the vitamin C content of the apple, as contrasted with apples from trees receiving no fertilizer, would be of interest. As a basis for this study, the Winesap variety, found by Potter (*9*) to be a good source of vitamin C, was selected since the minimum protective level had been established.

MATERIALS AND METHODS

In an attempt to obtain more definite information on the possible influence of applying complete fertilizers upon the vitamin C content of Winesap apples, fruit was obtained from fertilizer experimental plots of the Divisions of Horticulture and Agronomy, Washington Agricultural Experiment Station, in the Wenatchee district. The trees were 20 years old and were growing in a Cashmere gravelly coarse sandy loam (Kocher (*6*)). The orchard was in cover crop, consisting of a poor stand of alfalfa with considerable weed growth, and was irrigated by the rill or furrow system.

The fertilized trees during the years 1928 to 1931, inclusive, received annual applications per tree of a complete fertilizer as follows: 5.7 pounds of an equal mixture of ammonium sulphate and

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[2] Appreciation is expressed to Leila W. Hunt, professor of foods and nutrition, College of Home Economics, for helpful suggestions and aid in the nutritional phases of this study; to Prof. F. L. Overley of the Division of Horticulture, who was responsible for the application of the fertilizers and who harvested the apples utilized.

[3] Reference is made by number (italic) to Literature Cited, p. 373.

sodium nitrate, 11.7 pounds superphosphate, and 3.9 pounds of a mixture of equal parts of muriate of potash and sulphate of potash. These fertilizers were broadcast in December either on the ground surface or on the snow. The check trees received no fertilizer applications.

Comparable samples of Extra Fancy grade of the commercial size of Winesap apples, averaging 125 specimens per box, were harvested from both plots at the commercial stage of maturity, and immediately forwarded to Pullman, where they were retained at temperatures of 40° to 45° F. The vitamin C studies which were initiated shortly after the fruit was received, were conducted with guinea pigs, using essentially the technic of Sherman, LaMer, and Campbell (*10*).

The guinea pigs were observed in the laboratory for at least one week before beginning the experiment, in order to select normally growing animals. During this period the basal vitamin-C-free ration was fed, supplemented with vitamin-C-rich foods, to adjust the animals to the basal ration. This basal ration was that of Sherman, et al. (*10*) with several modifications:

	Per cent
Oats, sound whole grain, ground	58
Baked skim-milk powder (heated for 4 hours at 110° C.)	30
Butterfat, filtered and freshly prepared	10
Sodium chloride	1
Cod-liver oil	1
	100

Forty-two guinea pigs, averaging approximately 325 g [4] each were used for this experiment, the animals being divided as follows:

	Number of animals
Basal ration, plus orange juice (positive control)	6
Basal ration only (negative control)	6
Basal ration, plus apple:	
5 g fertilized Winesap apple	15
5 g nonfertilized Winesap apple	15

(Apple feedings made daily, 6 days per week.)

The animals were kept individually in galvanized screen cages with raised false bottoms. Fresh water and the basal ration were supplied daily.

The guinea pigs were observed daily for lameness, soreness, and enlargement of wrists, and were weighed on alternate days. The experimental periods used during 1930–31 were of 70 to 90 days' duration. Since Morgan et al. (*8*) presented data which show the adequacy of a shorter experimental period, the interval employed during 1931–32 was 56 days.

The positive-control animals were given orange juice as their source of vitamin C, this being fed at a level well above the generally accepted protection requirement.

The apple feedings were prepared each day following a definite technic. Vitamin C is easily destroyed by oxidation, as has been demonstrated by Kohman (*7*) and Kenney,[5] and other workers. Hence, a method was developed by which a uniform sample of apple pulp could be prepared quickly with minimum oxidation resulting.

[4] g is the abbreviation for gram or grams recently adopted by the Style Manual for U. S. Government printing.

[5] KENNEY, C. L. A STUDY OF THE THERMOSTABILITY OF VITAMIN C. 1926. (Diss., Columbia Univ.)

The apples were wiped, cored, quartered, and the skin surfaces scarified about one-eighth inch apart. The apple tissues were then ground, weighed, and fed to the animals immediately. Such a method produced a composite sample, with the skin and subepidermal tissues uniformly distributed. According to Bracewell, Kidd, West, and Zilva (*3*), these tissues contain the greatest concentration of vitamin C. Furthermore, the feedings were consumed by the animals before there was appreciable evidence of brown discoloration from oxidation.

At the close of the experimental period, autopsy studies were made on all animals, the Sherman scurvy score (*10*) being used to evaluate and record the findings. With this method of scoring, 0 indicated an absence of apparent evidence of scurvy while a score of 24 signified a condition of severe scurvy in all of the enumerated points of examination. The range from 1 to 24 was divided into four classes, with an interval width of 6. These classes designated the following states of scurvy: 1–6, mild; 7–12, moderate; 13–18, severe; and 19–24, very severe.

In expressing autopsy findings a negative sign indicated absence of scurvy, while 1 to 3 plus signs showed an increasing degree of severity of scurvy. It was thought that possible differences in vitamin C value between fruit of apple trees receiving complete fertilizers and apples from trees not fertilized would be more apparent when the animals were fed quantities slightly below the protective dose. Potter (*9*) found that Winesap apples, whether from fertilized or non-fertilized trees, when fed in 10 g amounts daily protected guinea pigs from scurvy and allowed them to gain in weight. The animals receiving the 5 g feedings, however, exhibited scurvy in all but a few cases. This smaller dosage of apple, therefore, seemed to offer the degree of protection where small differences in vitamin C content could be discerned.

EXPERIMENTAL RESULTS

The experimental data are shown in Table 1.

TABLE 1.—*Results of feeding Winesap apples from fertilized and from unfertilized trees to guinea pigs as the sole source of vitamin C*

Animal No.	Body weight				Duration of experiment	Autopsy findings [a]								Scurvy score	Final scorbutic condition
						Bony system				Hemorrhages					
	Initial	Maximum	Final	Average daily loss or gain		Teeth	Joints	Ribs	Bones	Ribs	Intestines	Joints	Muscles		
	Grams	*Grams*	*Grams*	*Grams*	*Days*										
POSITIVE CONTROLS FED BASAL DIET AND ORANGE JUICE															
2	300	421	421	+1.3	90	−	−	−	−	−	−	−	−	0	No scurvy.
4	297	448	448	+1.7	90	−	−	−	−	−	−	−	−	0	Do.
47	285	452	452	+2.4	70	−	−	−	−	−	−	−	−	0	Do.
48	295	546	546	+3.6	70	−	−	−	−	−	−	−	−	0	Do.
75	364	469	469	+1.9	56	−	−	−	−	−	−	−	−	0	Do.
82	331	501	493	+2.9	56	−	−	−	−	−	−	−	−	0	Do.
Average	312	473	471	+2.3	72	0	0	0	0	0	0	0	0	0	
NEGATIVE CONTROLS FED BASAL DIET ONLY															
5	310	310	179	−5.2	25	+++	++	+++	++	+++	+++	+++	++	21	Very severe.
11	297	297	215	−3.9	21	+++	+++	+	+++	+	+	+	+	14	Severe.
17	301	301	155	−5.6	26	+++	+++	+++	++	++	+++	++	++	20	Very severe.
18	290	290	152	−6.3	22	+++	+++	+++	+++	+	+++	++	++	20	Do.
43	281	281	142	−4.0	35	+++	+++	+++	+++	++	++	++	++	20	Do.
60	323	323	176	−5.7	26	++	+++	++	++	++	+	++	++	16	Severe.
Average	300	300	170	−5.1	26	2.8	2.8	2.5	2.5	1.8	2.2	2.0	1.8	19	

FED BASAL DIET AND 5 G OF WINESAP APPLE FROM FERTILIZED TREES

6	300	384	361	+0.7	90	−	−	−	−	−	−	−	−	0	No scurvy.
7	333	357	305	−.3	90	−	−	−	−	−	−	−	−	0	Do.
8	307	391	372	+.7	90	+	+	+	+	+	++	+	+	9	Moderate.
9	305	305	289	−.2	70	−	−	−	−	−	−	−	−	0	No scurvy.
10	301	301	204	−1.7	56	++	+	+	+	−	−	−	+	6	Mild.
36	290	301	179	−2.2	50	++	+	++	+	++	−	+	++	11	Moderate.
37	279	358	354	+.8	90	+	−	+	−	−	−	−	+	3	Mild.
56	336	336	229	−1.9	56	+	++	++	++	−	−	++	+++	13	Severe.
61	365	365	185	−3.5	52	++	++	++	++	−	−	+	+	10	Moderate.
64	399	412	343	−1.0	56	++	−	+	−	−	−	−	+	3	Mild.
65	337	347	203	−3.1	43	+	+	+	+	−	−	+	+	6	Do.
70	379	379	330	−.9	56	+	+	+	+	−	−	−	−	4	Do.
74	319	352	237	−1.5	56	+	++	+++	+	+	−	+	+	10	Moderate.
77	419	443	315	−1.9	56	+	++	++	++	−	−	++	+++	12	Do.
80	294	313	243	−.9	56	+	+	+	+	−	−	+	+	6	Mild.
Average	331	356	277	−1.1		1.1	0.9	1.2	0.9	0.3	0.2	0.7	1	6	

FED BASAL DIET AND 5 G WINESAP APPLE FROM UNFERTILIZED TREES

13	296	355	279	−0.2	90	++	++	+++	++	++	++	+++	++	18	Severe.
14	297	355	355	+.8	70	−	−	+	−	−	−	−	−	1	Mild.
15	289	470	457	+1.9	90	+	−	−	+	−	−	−	−	2	Do.
20	305	349	206	−1.5	68	+++	+	+	+	++	−	+	+++	11	Moderate.
41	282	326	200	−1.2	70	+++	+++	++	+++	+++	−	+++	++	19	Very severe.
46	294	294	185	−2.6	42	+++	+++	+++	+++	+++	−	+++	++	18	Severe.
53	300	302	195	−1.9	56	+++	+++	+	++	−	+	+	−	9	Moderate.
54	348	350	268	−1.4	56	++	+++	+	++	−	−	++	++	12	Do.
59	356	359	230	−2.3	56	+	++	++	+	+	−	++	+	10	Do.
63	381	412	328	−.9	56	+	−	?	−	−	−	−	−	1½	Mild.
68	345	351	237	−3.0	36	+	+	+	−	+	+	++	+	8	Moderate.
69	440	488	254	−4.1	45	++	++	+++	++	++	+	+	+	14	Severe.
72	350	352	296	−1.0	56	+	+++	+++	+	+	+	++	+	11	Moderate.
78	326	326	225	−1.8	56	++	+++	+	++	+	−	+++	+++	14	Severe.
79	376	376	289	−1.6	56	++	++	++	+	−	−	+	+++	11	Moderate.
Average	332	364	267	−1.6		1.6	1.6	1.5	1.4	1.1	0.4	1.6	1.4	10.6	

[a] Intensity of scurvy is indicated by plus signs, each having an arbitrary value of 1; minus signs indicate absence of symptoms.

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The positive-control animals lived throughout their experimental periods, and were protected from scurvy. The average weight gains for this group were 2.3 g daily. The negative-control group, however, lost 5.1 g daily, and had an average survival period of only 26 days. These negative animals showed a very severe scorbutic condition at autopsy as the high average scurvy score of 19 indicated. Their life period on the scorbutic basal diet alone corresponds very closely with the data of Sherman, LaMer, and Campbell (*10*).

In Table 1, data are also presented for the guinea pigs that were fed 5 g amounts of the Winesap apples from trees receiving applications of the complete fertilizer. Three animals died from scurvy before the end of their experimental period. The average scurvy score for the group was 6, which indicated a mild scorbutic condition. The average daily weight loss was 1.1 g.

The records for the guinea pigs that were fed 5 g of nonfertilized Winesap apples as their source of vitamin C are likewise shown in Table 1. In this group four animals died before the experimental period terminated, and had marked scorbutic lesions. Most of the animals lost weight (an average loss of 1.6 g daily) and those surviving the experimental period with only three exceptions showed marked evidences of scurvy at post-mortem examinations. The average scurvy score for this group was 10.6 which would give the group an average rating of moderate scurvy.

DISCUSSION OF RESULTS

Bracewell, Wallace, and Zilva (*4, p. 146*) summarized their findings concerning the relation of potency of vitamin C to nitrogen content of apples as follows:

> King Edwards containing about 0.0307% of nitrogen were about 1.5 times as potent antiscorbutically as apples of this variety containing about 0.0387% of nitrogen. No significant disparity in the vitamin C content was found between Bramley's Seedlings containing high and low quantities of nitrogen.

In view of these results it would have been of interest to know the nitrogen content of the fertilized and nonfertilized Winesap apples employed in the experiment herewith reported. Such analyses, however, are not available. According to Gourley and Hopkins (*5*), and Aldrich (*1*), the application of nitrogen-carrying fertilizers tends to increase the nitrogen content of the apples of trees so fertilized. It is of course possible that some one of the other elements in the complete fertilizer applied, rather than nitrogen, may have been responsible for differences observed in vitamin C potency between the fertilized and unfertilized Winesap apples, and as herewith reported. It is also possible that the effect may have been an indirect one rather than any increase in the actual mineral content of the apple.

CONCLUSIONS

The fact that 60 per cent of the animals receiving apples from fertilized trees were protected or developed only mild scurvy, while with the animals fed apples from nonfertilized trees not one was protected and 80 per cent developed moderate to very severe scurvy, is significant.

From the data submitted, Winesap apples from trees receiving applications of a complete fertilizer appeared to be a better source of vitamin C than apples from trees not so fertilized, when fed at the

5 g level. This should not be interpreted as indicating that Winesap apples are an unsatisfactory source of vitamin C unless from trees receiving applications of a complete fertilizer. Potter (*9*) has found that when fed at the 10 g level, differences were not apparent between fruit from fertilized and from nonfertilized trees.

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YIELD AND CHEMICAL COMPOSITION OF CERTAIN PASTURE CROPS, FERTILIZED AND UNFERTILIZED [1]

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INTRODUCTION

The high yields of pasture herbage obtainable in the valleys of western Washington make it desirable to secure data relative to the value of adapted grasses, and the changes that occur as a result of fertilizer applications or improved methods of management.

Unpublished data secured by McCollam [2] in 1928 and by the author in 1929 showed that yields ranging from 6,000 to 10,000 pounds of dry matter per acre could be obtained from pasture crops grown on moist bottom-land soil, when the crops were cut at intervals of approximately a month. It was found, however, that with a growing period of a month, the grasses reached a comparatively advanced stage of maturity. In the work here reported, a 14-day growth period was allowed between cuttings in place of the monthly period previously employed, since it was desired to obtain information relative to the composition and yield of pasture crops in the more immature stages.

WORK OF PREVIOUS INVESTIGATORS

McCollam,[2] at the Western Washington Experiment Station, in 1928 recorded yields on a number of pasture crops growing on a muck soil well supplied with moisture. A bottom-land mixture consisting of adapted grasses and clovers recommended for western Washington (*8*),[3] cut on the specified dates, produced the following yields of dry matter in pounds per acre: April 27, 2,375.7 pounds; June 5, 2,544.6 pounds; July 9, 2,252.9 pounds; August 4, 1,659.4 pounds; and August 25, 861 pounds. The total yield for the season was 9,693.6 pounds of dry matter per acre. During the same period Italian ryegrass produced 8,697.8 pounds of dry matter per acre, and white clover 6,685.8 pounds of dry matter per acre.

Woodman, Norman, and Bee (*14*) analyzed samples of a mixed grass cut at intervals of three weeks from April to October, 1929. They found that the average protein content of the grass cut in this manner was 21.14 per cent, as compared with 23.48 per cent for fortnightly cuttings made in 1927 and 24.74 per cent for weekly cuttings made in 1925.

Ellenberger, Newlander, and Jones (*2*) secured from frequently clipped pasture plots (1925 and 1926) an average yield of 1,748 pounds of dry matter per acre. The average composition of the clippings was: Crude protein, 20.30 per cent; crude fiber, 18.69 per cent; crude ash, 11.68 per cent; nitrogen-free extract, 46.03 per cent;

[1] Received for publication May 4, 1932, issued March, 1933.

[2] MCCOLLAM, M. E., unpublished data.

[3] Reference is made by number (italic) to Literature Cited, p. 386.

ether extract, 3.3 per cent; calcium, 0.816 per cent; and phosphorus, 0.32 per cent. They concluded (*2, p. 65*) "the nutrients in closely grazed pasture more nearly resemble a concentrate than a roughage."

Hopper and Nesbitt (*7*) were able to maintain a high percentage of protein throughout the season in pure stands of bromegrass, slender wheatgrass, and crested wheatgrass, by cutting twice monthly.

Shutt, Hamilton, and Selwyn (*10*) in 1927 obtained an average protein content of meadow foxtail (*Alopecurus* sp.) of 21.20 per cent (dry-matter basis) for plots cut weekly, 18.60 per cent for plots cut fortnightly, and 17.17 per cent for plots cut every three weeks. The seasonal yields of dry matter for cuttings at intervals of 1, 2, and 3 weeks were 2,918, 3,344, and 4,304 pounds, respectively.

Archibald and Nelson (*1*) found that an application of complete fertilizer followed by later applications of nitrogenous fertilizer on a rotationally grazed pasture decreased considerably the percentage of dry matter in the grass, and decreased slightly the percentage of crude fiber and calcium in the dry matter. The percentage of nitrogen, phosphorus, and ether extract was increased, the nitrogen markedly so.

Greenhill (*5*) found that the average protein content of rotationally grazed pasture was increased from 22.6 per cent to 25.3 per cent by intensive fertilization with nitrogenous fertilizers.

Enlow and Coleman (*3, p. 852*) concluded: "The protein content of pasture in a grazed condition can be increased and maintained at a somewhat higher level than ordinary by frequent light applications of a nitrogen fertilizer."

METHOD

The plots used in this experiment are located on a low-lying tract of valley land belonging to the Puget series (sandy loam). During the winter months the water table lies approximately 18 inches below the surface. Following periods of exceptionally heavy rains, however, the water table frequently comes to within a few inches of the surface for short periods. In the dry months of late summer, the water table falls to a depth in excess of 3 feet. The soil used in this experiment is typical of considerable areas of alluvial soil lying in the valleys of the Puget Sound Basin. While the soil is productive, crops are often limited in their growth by lack of moisture during the summer and fall months.

The area had previously been in pasture for six years, and had received during that period, one or two light applications of barnyard manure. The latest manure application had been made during the winter of 1928–29.

The land was plowed during the winter previous to seeding the plots. The area was disked and harrowed a number of times before the final preparation of the seed bed. After the ground had been thoroughly prepared, twelve $\frac{1}{200}$-acre plots were staked out. Six of these plots received the following fertilizers:[4] N, 45 pounds; P_2O_5, 87.5 pounds; and K_2O, 100 pounds per acre.

The carriers employed were sodium nitrate, superphosphate, (17.5 per cent P_2O_5), and potassium chloride. Hydrated lime ($Ca(OH)_2$) at the rate of 2,000 pounds per acre was also applied to

[4] The application of fertilizers and hydrated lime was made at rates suggested by Henry Holtz, soils section, department of agronomy, State College of Washington.

the plots receiving the fertilizer. The fertilizers applied just before the plots were seeded were worked into the topsoil with a hand rake.

Two pasture crops (Italian ryegrass and white clover) seeded alone, and one mixture were used in this experiment. The mixture was the bottom-land mixture recommended for western Washington by McCollam (*8*). This mixture was made up of grasses and clovers in the following proportions by weight: Italian ryegrass, 3; English ryegrass, 3; meadow fescue, 4; tall meadow oatgrass, 2; seaside bent grass, 3; common white clover, 4; red clover, 2; and alsike clover, 1. Duplicate $\frac{1}{200}$-acre plots of each crop were seeded on both fertilized and unfertilized areas, between March 15 and 20, 1930.

Rapid growth was made on each plot, and on May 12, a preliminary cutting of the Italian ryegrass and bottom-land mixture was made. The preliminary cutting of the white clover was made on May 19. A lawn mower was used throughout the season for cutting the plots. The cutting bar of the lawn mower was set to cut at a height of 1¼ inches.

The preliminary cutting was discarded. Succeeding cuttings of the plots were made at regular intervals of 14 days. The green material from the duplicate $\frac{1}{200}$-acre plots of each crop was combined at the time of cutting and thereafter was considered as the yield from one-hundredth of an acre. The regular biweekly cuttings were continued through December, 1930. Immediately after being cut, the fresh grass was weighed in the laboratory, to obtain the green weight, and thoroughly mixed. Duplicate 100-g[5] samples were then taken from each lot. These samples were put in muslin bags and dried[6] until no further change in weight occurred.

The average percentage of dry matter of the duplicate 100-g samples was taken as the percentage of dry matter for the entire sample. The remainder of the material from each cutting was also dried and preserved for analysis. One sample for analysis was obtained each month from each plot. This sample was prepared by thoroughly mixing all the dry material from cuts made on one plot during one month. A portion of this material was then analyzed.[7]

PRESENTATION OF DATA AND DISCUSSION OF RESULTS

Table 1 shows that the year 1930 was unusually dry, the total rainfall being 9.33 inches less than the average for the previous 10 years.[8] A fair amount of rain fell until June 7, but from that date until late September, the rainfall was far below normal. From June 8 to September 26, a period of over three and one-half months, the total rainfall was only 1.05 inches. The rainfall was 0.84 inch and 0.72 inch above normal in May and June, respectively, but that for July, August, and September was 0.50, 1.06, and 0.97 inches, respectively, below normal. On September 27 a heavy rain fell, and thereafter the rainfall was more abundant. Table 1 indicates that the precipitation for July, August, and September was very unfavorable to the growth of pasture grasses.

[5] g is the abbreviation for gram or grams recently adopted by the Style Manual for United States Government printing.

[6] The temperature of drying was approximately 85° C. It was impossible with the oven used, to keep the temperature entirely uniform; the range was from 80° to 95°.

[7] The samples were analyzed by Clarence Kaseline in the laboratories of the division of chemistry of the agricultural experiment station.

[8] From the records obtained at the U. S. Weather Bureau station located on the grounds of the Western Washington Station.

Table 1 also shows that the temperature during the 1930 growing season, as compared with the average for the previous 10-year period, was favorable to growth. The temperature during July, August, and September averaged higher than that during May and June. In spite of this fact, production decreased during July, August, and September because of unfavorable moisture conditions.

TABLE 1.—*Rainfall and mean monthly temperatures for 1930, as compared with averages for the previous 10-year period, 1920–1929*

RAINFALL (INCHES)

	January	February	March	April	May	June	July	August	September	October	November	December	Annual
Average for 1920–1929	5.67	3.38	3.50	2.54	2.18	1.06	0.50	1.20	2.00	3.68	4.38	5.87	35.96
1930	2.15	5.46	2.97	2.22	3.02	1.78	0	.14	1.03	2.43	3.26	2.17	26.63
Deviation in 1930	−3.52	2.08	−.53	−.32	.84	.72	−.50	−1.06	−.97	−1.25	−1.12	−3.70	−9.33

MEAN MONTHLY TEMPERATURES (°F.)

Average for 1920–1929	38.6	40.8	44.8	48.6	54.5	60.1	63.6	56.4	51.8	46.3	44.2	39.5	45.3
1930	29.0	43.4	46.2	51.8	53.8	59.0	62.8	64.8	58.8	48.8	43.0	39.4	50.1
Deviation in 1930	−9.6	2.6	1.4	3.2	−.7	−1.1	−.8	8.4	7.0	2.5	−1.2	−.1	4.8

Table 2 shows that the highest yields of dry matter in all plots were obtained during June. Beginning late in June or early in July, there is a decrease in the production of all plots to the end of the year. There seems to be little difference between the fertilized and the unfertilized plots in this respect, although the production of the fertilized plots was usually at a higher level.

TABLE 2.—*Yield of dry matter in pounds per acre, and gain or loss in yield per acre produced by fertilizer treatment of Italian ryegrass, bottom-land mixture, and white clover cut at 14-day intervals from fertilized and unfertilized plots*

Date of cutting	Italian ryegrass			Bottom-land mixture			White clover			
	Fertilized	Unfertilized	Gain or loss	Fertilized	Unfertilized	Gain or loss	Date of cutting	Fertilized	Unfertilized	Gain or loss
May 26	1,080.6	703.0	377.6	1,093.1	820.3	272.8	June 2	1,218.3	789.7	428.6
June 9	1,472.6	973.8	498.8	1,595.7	1,398.9	196.8	June 16	879.0	794.0	85.0
June 23	849.3	677.2	172.1	1,029.5	908.0	121.5	June 30	1,145.3	779.3	366.0
July 7	878.6	777.8	100.8	907.6	899.7	7.9	July 14	733.0	598.1	134.9
July 21	674.5	775.6	−101.1	656.8	678.7	−21.9	July 28	629.3	570.6	58.7
Aug. 4	542.3	359.5	182.8	482.6	374.5	108.1	Aug. 11	666.0	566.5	99.5
Aug. 18	544.0	483.0	61.0	396.3	310.7	85.6	Aug. 25	411.7	393.7	18.0
Sept. 1	390.3	281.5	108.8	294.2	229.1	65.1	Sept. 8	304.2	389.3	−85.1
Sept. 15	385.0	277.1	107.9	216.5	226.6	−10.1	Sept. 22	330.7	215.2	115.5
Sept. 29	323.1	187.8	135.3	239.6	163.5	76.1	Oct. 6	158.4	140.4	18.0
Oct. 13	250.0	148.6	101.4	218.7	155.2	63.5	Oct. 20	128.2	77.4	50.8
Oct. 27	156.8	144.3	12.5	169.2	131.4	37.8				
Nov. 10	124.9	134.6	−9.7	154.0	110.3	43.7				
Nov. 24	63.0	58.9	4.1	71.2	56.9	14.3				
Dec. 8	26.0	22.8	3.2	32.6	19.9	12.7				
Dec. 22	57.8	36.3	21.5	61.2	38.7	22.5				
Total	7,818.8	6,041.8	1,777.0	7,618.8	6,522.4	1,096.4		6,604.1	5,314.2	1,289.9

On the white-clover plots, although they were seeded to a pure stand, there was at all times a small percentage of grass present in the clippings. During the late spring and summer months this consisted to a large extent of annual bluegrass (*Poa annua* L.), but during the fall and winter months some velvet grass (*Holcus lanatus* L.) also invaded the clover plots. While the white clover was growing rapidly, the percentage of grass was small, but as the growth of the clover decreased, the grass became more and more apparent, until during November and December the samples from the plots consisted mostly of foreign species. For this reason, the data on white clover for November and December are not presented.

The single fertilizer treatment applied early in the spring was not instrumental in maintaining a uniform production over the growing season. In fact, the high production obtained during the late spring months was still further increased by the fertilizer application. This was to be expected since the fertilizer application contained a considerable quantity of nitrogen. The fertilizer was instrumental in maintaining, with few exceptions, a somewhat higher production than was obtained on the unfertilized plots.

The percentages of dry matter of the different cuts are not presented in full, since it was often necessary to cut the plots when the grass was wet with rain or dew. For this reason, these data are not considered to represent exact values. The total dry weights for the season and the seasonal average percentages of dry matter are presented in Table 3.

TABLE 3.—*Total dry weights in pounds per acre of, and average percentages of dry matter in, Italian ryegrass, bottom-land mixture, and white clover harvested from fertilized and unfertilized plots*

Treatment	Italian ryegrass		Bottom-land mixture		White clover	
	Dry weight	Dry matter	Dry weight	Dry matter	Dry weight	Dry matter
	Pounds	*Per cent*	*Pounds*	*Per cent*	*Pounds*	*Per cent*
Fertilized	7,818.8	16.44	7,618.8	16.10	6,604.1	13.23
Unfertilized	6,041.8	16.72	6,522.4	15.82	5,314.2	14.90

The average percentage [9] (weighted average) of crude protein for the season as presented in Table 4 shows that there has been no great increase in the protein content as a result of a single fertilizer application. The greatest increase for the season, 1.13 per cent, was obtained in the white clover. The bottom-land mixture, on the other hand, showed an actual decrease of 1.05 per cent in protein content on the fertilized plot. It should be noted, however, that there is in all three crops, an increase in the percentage of protein on the fertilized plots for the first two or three months, indicating a possible influence of the nitrogen application. This suggests that frequent applications of nitrogenous fertilizers might result in a materially increased percentage of protein. Enlow and Coleman (*3*) found this to be the case.

[9] The average percentage for the season of crude protein, etc., was obtained by computing the production for each cutting and comparing the total for the season with the total yield of dry matter.

TABLE 4.—*Percentage constitution on a dry-matter basis of Italian ryegrass, bottom-land mixture, and white clover, harvested in different months from fertilized and from unfertilized plots*

CRUDE PROTEIN

Month	Italian ryegrass		Bottom-land mixture		White clover	
	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized
May	23.62	22.50	28.58	27.12		
June	22.98	21.62	26.06	25.62	29.01	27.00
July	20.56	21.47	21.98	23.75	30.36	28.81
August	21.47	23.03	20.98	28.95	30.20	29.74
September	26.14	26.05	25.27	26.67	28.31	30.24
October	29.50	28.43	28.55	35.36	33.98	33.04
November	31.47	31.86	30.90	25.85		
December	28.11	30.18	29.29	30.35		
Weighted average	23.42	23.17	25.23	26.28	29.63	28.50

CRUDE FIBER

May	14.78	14.36	16.26	15.94		
June	20.75	18.50	19.24	18.79	15.70	14.08
July	19.70	19.00	17.97	18.19	14.54	14.20
August	20.71	18.69	20.00	17.14	14.87	14.03
September	18.46	17.17	18.29	15.86	13.89	13.58
October	16.24	15.15	15.70	14.76	12.93	13.82
November	15.87	15.17	15.91	15.35		
December	11.98	12.36	12.26	12.83		
Weighted average	18.94	17.68	18.18	17.52	15.03	14.03

ETHER EXTRACT

May	3.52	3.03	2.95	2.80		
June	3.69	3.13	3.52	3.00	3.52	3.49
July	3.49	3.52	2.92	3.16	3.82	3.67
August	3.70	3.74	2.86	2.82	3.15	3.69
September	3.77	3.73	3.86	4.76	7.48	3.77
October	3.64	4.85	4.74	4.79	5.49	4.19
November	5.45	4.63	5.19	4.99		
December	5.30	5.29	5.29	4.98		
Weighted average	3.69	3.53	3.40	3.31	3.99	3.63

CRUDE ASH

May	11.02	9.76	13.30	11.92		
June	13.00	11.67	13.20	12.39	11.51	10.62
July	13.04	12.38	12.16	12.01	10.01	9.34
August	13.30	12.92	12.45	11.88	9.18	8.25
September	15.97	14.13	13.53	12.53	9.14	11.10
October	14.09	13.29	12.40	12.45	10.14	10.55
November	12.57	13.39	12.10	12.48		
December	9.22	16.55	9.30	9.07		
Weighted average	13.20	12.29	12.83	12.17	10.53	9.96

NITROGEN-FREE EXTRACT

May	47.06	50.35	38.91	42.22		
June	39.58	45.08	37.98	40.20	40.26	44.81
July	43.21	43.63	44.97	42.89	41.27	43.98
August	40.82	41.62	43.71	39.21	42.60	44.29
September	35.66	38.92	39.05	40.18	41.18	41.31
October	36.53	38.28	38.61	32.64	37.46	38.40
November	34.64	34.95	35.90	41.33		
December	45.39	35.62	43.86	42.77		
Weighted average	40.75	43.33	40.36	40.72	40.82	43.88

TABLE 4.—*Percentage constitution on a dry-matter basis of Italian ryegrass, bottom-land mixture, and white clover, harvested in different months from fertilized and from unfertilized plots*—Continued

CALCIUM

Month	Italian ryegrass		Bottom-land mixture		White clover	
	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized
May	0.54	0.41	0.69	0.67		
June	.62	.56	.64	.69	1.32	1.06
July	.58	.59	.58	.67	1.22	1.13
August	.57	.63	.54	.65	1.02	1.00
September	.65	.64	.59	.63	.96	.96
October	.64	.50	.51	.56	.84	.76
November	.60	.46	.44	.42		
December	.51	.44	.43	.34		
Average for season	.596	.561	.602	.655	1.194	1.040

PHOSPHOROUS

May	0.54	0.47	0.55	0.65		
June	.64	57	.63	.60	0.68	0.63
July	.60	.60	.51	.52	.49	.50
August	.61	.58	.51	.51	.47	.48
September	.63	.56	.58	.50	.46	.45
October	.65	.60	.54	.60	.52	.52
November	.69	.65	.63	.70		
December	.57	.58	.70	.63		
Average for season	.612	.569	.570	.569	.575	.548

The increased percentage of protein found in the Italian ryegrass (and to a lesser degree in the bottom-land mixture) during the later months is probably due to a less advanced stage of maturity reached by these crops in the 14-day interval at this period of the year.

Table 4 shows that there is in the Italian ryegrass and bottom-land mixture a considerable decrease in the percentage of crude fiber from August to December. This decrease is undoubtedly due to the fact that the grass, although cut at equal intervals throughout the season, was actually cut at a more immature stage in the later months than in the months of rapid growth. The fact that the Italian ryegrass, during the period of rapid growth, often showed seed heads before the end of the 14-day period, supports this view.

The data show that there has been a rather consistent increase in the percentage of fiber in the fertilized crops over that in the unfertilized crops. A more advanced stage of maturity attained in the 14-day period on the fertilized plots may be responsible for this.

Taking the average for the whole season, there has been a slight increase in the ether extract content of the pasture crops as a result of the fertilizer treatment. (Table 4.) This increase is in no case large but is found in all three crops. (This is in agreement with the results obtained by Archibald and Nelson (*1*), who found an increase in the percentage of ether extract as a result of fertilization.)

There are, however, a number of cases where the percentage of ether extract in the unfertilized crops is greater than that in the fertilized crops. For this reason the comparatively insignificant increases for the season shown in Table 4 can not be considered conclusive.

In all three crops, there is an increase in the percentage of ether extract as the season advances.

The figures in Table 4 show an appreciable increase in the percentage of crude ash on all fertilized plots for the season. These figures, however, represent the percentage of crude ash rather than silica-free ash.

The percentages of nitrogen-free extract of the different crops are presented in Table 4 only for comparison. While appreciable differences exist between the fertilized and unfertilized crops, these differences are not considered significant, since the data were secured by subtraction.

Table 4 indicates that no significant changes in calcium content have occurred as a result of the fertilizer treatment except in the white clover during June and July. The greatest difference lies between the different crops rather than in seasonal differences in a single crop, the white clover having a decidedly higher calcium content than the Italian ryegrass. The bottom-land mixture ranks between the other two grasses in calcium content, showing the effect of the clover in the mixture.

There is a decided drop in the calcium content of the white clover during the fall months, and the bottom-land mixture shows a similar decline. While this is apparently a seasonal change in the calcium content of the crop, it is probably due, at least in part, to the gradual decrease in the proportion of clover on these plots, and the resulting increase in the proportion of grass (by invasion on the clover plots). From the data shown it would seem that the percentage of calcium could be increased most readily by increasing the percentage of clover in a pasture crop.

An indication as to the amount of total calcium contained in soil of the type used in this experiment is found in the work of Holtz (*6*) in 1930. Holtz found an average of 1.855 per cent total calcium in the soils of the Puget series. Two samples of Puget sandy loam obtained from the western Washington station average 2.21 per cent total calcium.

The percentage of phosphorus recovered in the pasture cuttings, under the conditions of the experiment, seems to be even less affected than the percentage of calcium by fertilizer applications or seasonal changes in production. While variations occur between different cuttings, significant differences are not apparent, either between fertilized and unfertilized plots, or between different crops. If any difference exists it is shown in the white clover and bottom-land mixture by the slight lowering of the phosphorus content during the dry summer months. The percentages of phosphorus, however, are in all cases comparatively high, as a comparison with the results obtained by other investigators will show. The percentages of phosphorus found in pasture grass by various investigators are:

Investigator	Percentage of phosphorus
Archibald and Nelson (*1*)	0.28 to 0.33.
Ellenberger, Newlander, and Jones (*2*)	0.32 average.
Forbes, Whittier, and Collison (*4*)	0.168 to 0.349 (1908–1909).
Rigg and Askew (*9*) (from pastures giving good results with livestock).	0.39 to 0.56 (0.90 to 1.29 P_2O_5).
Woodman, Bee, and Griffith (*11*) (dried-grass cakes).	0.39 to 0.43 (0.90 to 1.00 P_2O_5).
Woodman and Evans (*13*) (mineral-deficient forage).	0.09 to 0.11 (0.21 to 0.27 P_2O_5).
Western Washington Station	0.45 to 0.70.

Holtz (*6*) found that soils of the Puget series averaged 0.0285 per cent available phosphorus. Two samples of Puget sandy loam from the western Washington station averaged 0.0444 per cent available phosphorus. This high percentage of available phosphorus probably accounts for the high percentage of phosphorus found in the crops studied, and the failure of the phosphorus application to increase the percentage of phosphorus in the herbage.

The figures showing the quantities of phosphorus per acre recovered from the different plots are quite striking. These figures are presented in Table 5.

TABLE 5.—*Phosphorus (pounds per acre) found in Italian ryegrass, bottom-land mixture, and white clover, from fertilized and unfertilized plots, as compared with the quantity applied in fertilizer*

Crop	Treatment	Yield of dry matter, per acre	Phosphorus in dry matter	Phosphorus contained in crops	Phosphorus per acre applied in fertilizer	Phosphorus (P_2O_5) contained in crops
		Pounds	*Per cent*	*Pounds*	*Pounds*	*Pounds*
Italian ryegrass	Fertilized	7,818.8	0.612	47.88	38.23	109.59
	Unfertilized	6,041.8	.569	34.41	----------	78.76
Bottom-land mixture	Fertilized	7,618.8	.570	43.45	38.23	99.45
	Unfertilized	6,522.4	.569	37.14	----------	85.01
White clover	Fertilized	6,604.1	.575	37.97	38.23	86.91
	Unfertilized	5,314.2	.548	29.14	----------	66.07

On the fertilized plots (except the white clover) more phosphorus was taken from the soil than was applied to the soil in the fertilizer. Even on the unfertilized plots, the phosphorus taken up by the crop was equivalent to a heavy application of phosphate fertilizer. While under actual pasture conditions a certain amount of phosphorus is returned to the soil in the manure, it is evident that under conditions of heavy yield, the pasture crop makes a heavy drain on the phosphorus resources of the soil.

The figures in Table 6 show the influence of clover on the calcium-phosphorus ratio. Under the conditions of the experiment, the presence or absence of clover has apparently had a much greater influence on the relative amounts of calcium and phosphorus in the dry matter than had the fertilizer treatment.

TABLE 6.—*Calcium-phosphorus ratios of dry matter of pasture crops cut at 14-day intervals from fertilized and from unfertilized plots*

Treatment	Italian ryegrass	Bottom-land mixture	White clover
Fertilized	1:1.014	1:0.94	1:0.48
Unfertilized	1:1.026	1: .86	1: .52

The data in Table 7 show that the yield of crude protein on the fertilized plots was generally higher than the yield on the unfertilized plots. This increase was due, however, largely to the increased yield of dry matter on the fertilized plots rather than to an increased percentage of protein in the dry matter. The largest increase in the percentage of protein due to fertilization (1.13 per cent in the white

clover) is comparatively insignificant. It is evident, therefore, that the increase in the yield of dry matter as a result of a fertilizer application, is a more important factor than an increase in the protein content. This is especially true since the nutritive ratio of immature grass is already comparatively narrow, as shown by the work of Woodman, Blunt, and Stewart (*12*) and Ellenberger, Newlander, and Jones (*2*).

TABLE 7.—*Yield of crude protein in pounds per acre, and gain or loss in yield produced by fertilizer treatment of Italian ryegrass, bottom-land mixture, and white clover cut at 14-day intervals from fertilized and unfertilized plots*

Date of cutting	Italian ryegrass			Bottom-land mixture			White clover			
	Fertilized	Unfertilized	Gain or loss	Fertilized	Unfertilized	Gain or loss	Date of cutting	Fertilized	Unfertilized	Gain or loss
May 26	255.23	158.17	97.06	312.40	222.46	89.94	June 2	353.42	213.21	140.21
June 9	338.40	210.53	127.87	415.83	358.39	57.44	June 16	254.99	214.38	40.61
June 23	195.16	146.41	48.75	268.28	232.62	35.66	June 30	332.25	210.41	121.84
July 7	180.64	166.99	13.65	199.49	213.67	−14.18	July 14	222.53	172.31	50.22
July 21	138.67	166.52	−27.85	144.36	161.19	−16.83	July 28	191.05	164.38	26.67
Aug. 4	116.43	82.79	33.64	101.24	108.41	−7.17	Aug. 11	201.13	168.47	32.66
Aug. 18	116.79	111.23	5.56	83.14	89.94	−6.80	Aug. 25	124.33	117.08	7.25
Sept. 1	102.02	73.33	28.69	74.34	61.10	13.24	Sept. 8	86.11	117.72	−31.61
Sept. 15	100.63	72.18	28.45	54.70	60.43	−5.73	Sept. 22	93.62	65.07	28.55
Sept. 29	84.45	48.92	35.53	60.54	43.60	16.94	Oct. 6	53.82	46.38	7.44
Oct. 13	73.75	42.24	31.51	62.43	54.87	7.56	Oct. 20	43.56	25.57	17.99
Oct. 27	46.25	41.02	5.23	48.30	46.46	1.84				
Nov. 10	39.30	42.88	−3.58	47.58	28.51	19.07				
Nov. 24	19.82	18.76	1.06	22.00	14.70	7.30				
Dec. 8	7.30	6.88	.42	9.54	6.03	3.51				
Dec. 22	16.24	10.95	5.29	17.92	11.74	6.18				
Total	1,831.08	1,399.80	431.28	1,922.09	1,714.12	207.97		1,956.81	1,514.98	441.83

In comparing the quantity of nitrogen actually applied on the fertilized plots with the excess of nitrogen (over the unfertilized crops) recovered in the dry matter of the fertilized crops (Table 8), it is interesting to note that in the Italian ryegrass and white clover, the amount of this excess nitrogen is actually much greater than the amount of nitrogen applied in the fertilizer.

TABLE 8.—*Excess nitrogen (pounds per acre) in Italian ryegrass, bottom-land mixture, and white clover, from fertilized plots, (over unfertilized plots) as compared with the quantity applied in fertilizer*

Crop	Excess protein (over unfertilized crops) contained in fertilized crops	Excess nitrogen (over unfertilized crops) contained in fertilized crops	Nitrogen applied per acre	Excess nitrogen contained in fertilized crop (over unfertilized crop) expressed as percentage of nitrogen applied as fertilizer
Italian ryegrass	431.28	69.00	45	153.33
Bottom-land mixture	207.97	33.27	45	73.93
White clover	441.83	70.69	45	157.09

SUMMARY

Studies on the yield of Italian ryegrass, bottom-land mixture, and white clover during the 1930 season made by cutting plots of these crops at regular intervals of 14 days, show that a period of heavy production during May and June was followed by a constant drop in the production for the remainder of the season.

The total yields of dry matter in pounds per acre for the season of the different crops are as follows: Italian ryegrass, fertilized, 7,818.8; unfertilized, 6,041.8; bottom-land mixture, fertilized, 7,618.8; unfertilized 6,522.4; and white clover, fertilized 6,604.1; unfertilized 5,314.2.

The percentages of crude protein for the season of the different crops are: Italian ryegrass, fertilized, 23.42; unfertilized, 23.17; bottom-land mixture, fertilized, 25.23, unfertilized, 26.28; and white clover, fertilized, 29.63, unfertilized 28.50.

The yields of dry matter and crude protein are, with few exceptions, higher through the season in the fertilized crops.

A high percentage of crude protein is maintained throughout the season by cutting pasture crops at 14-day intervals. The percentage of crude protein shows a tendency to increase during the fall months. This is believed to be due to greater immaturity of the crop at the time of cutting.

Under the conditions of low rainfall occurring in 1930 the presence or absence of clover exerts a greater influence on the protein content than does a single application of fertilizer made at the beginning of the season.

The fiber content of the fertilized crops is consistently higher than that of the unfertilized crops.

The increase in the yield of protein as a result of the fertilizer treatment is 431.28 pounds per acre for the Italian ryegrass, 207.97 pounds for the bottom-land mixture, and 441.83 pounds for the white clover. The use of fertilizer containing 45 pounds of nitrogen per acre resulted in an increased recovery of nitrogen, amounting to 69.00, 33.27, and 70.69 pounds for the Italian ryegrass, bottom-land mixture, and white clover, respectively. As compared with the nitrogen applied, the increases in nitrogen recovery resulting from the fertilizer application amount to 153.33, 73.93, and 157.09 per cent, respectively, for the three crops.

The percentage of ether extract and crude ash are higher in the fertilized crops than in the unfertilized crops. The percentage of ether extract shows a considerable tendency to increase during the fall months.

No marked changes in calcium content occur as a result of the fertilizer treatment. The clover contains by far the highest percentage of calcium, while the bottom-land mixture is intermediate between Italian ryegrass and white clover in this respect. Clover appears to be a more important factor in determining calcium content of a forage crop than does the fertilizer.

The calcium-phosphorus ratio appears to be influenced by the presence or absence of clover. The influence of the fertilizer on the calcium-phosphorus ratio is insignificant. The recovery of phosphorus in high-yielding pasture crops is equivalent to a heavy application of phosphate fertilizer.

Under the conditions of the experiment, the increase in the yield of dry matter is found to be a more important result of the fertilizer treatment than is any increase in the percentage of protein, calcium, or phosphorus.

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